ARID1B is a specific vulnerability in ARID1A-mutant cancers

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Recent studies have revealed that ARID1A, encoding AT-rich interactive domain 1A (SWI-like), is frequently mutated across a variety of human cancers and also has bona fide tumor suppressor properties. Consequently, identification of vulnerabilities conferred by ARID1A mutation would have major relevance for human cancer. Here, using a broad screening approach, we identify ARID1B, an ARID1A homolog whose gene product is mutually exclusive with ARID1A in SWI/SNF complexes, as the number 1 gene preferentially required for the survival of ARID1A-mutant cancer cell lines. We show that loss of ARID1B in ARID1A-deficient backgrounds destabilizes SWI/SNF and impairs proliferation in both cancer cells and primary cells. We also find that ARID1A and ARID1B are frequently co-mutated in cancer but that ARID1A-deficient cancers retain at least one functional ARID1B allele. These results suggest that loss of ARID1A and ARID1B alleles cooperatively promotes cancer formation but also results in a unique functional dependence. The results further identify ARID1B as a potential therapeutic target for ARID1A-mutant cancers.

To search for specific vulnerabilities created by ARID1A mutation, we used data from Project Achilles, a large-scale project focused on identifying essential genes in a wide panel of cancer cell lines using genome-scale loss-of-function genetics1. We compared 18 ARID1A-mutant cell lines to 147 cell lines wild type for ARID1A (hereafter referred to as wild-type). Of 9,050 genes interrogated, ARID1B scored as the top candidate preferentially required for the growth of ARID1A-mutant cancer cell lines ($P = 7.366 \times 10^{-6}$, false discovery rate (FDR) < 0.001) (Fig. 1a and Supplementary Fig. 1a). Vulnerability to ARID1B depletion was even more pronounced in the large subset of cell lines that contained inactivating ARID1A mutations (rather than missense mutations) (Fig. 1b), which suggests that ARID1B is specifically essential for cell lines that carry inactivating mutations in ARID1A (Supplementary Discussion). To further evaluate this finding, we examined the effects of the individual ARID1B-targeting shRNAs (ARID1B shRNAs). Three of the four ARID1B shRNAs passed the Achilles quality control metrics2. These scored as the number 1 ($P = 1.211 \times 10^{-6}$, FDR < 0.001), 4 ($P = 1.211 \times 10^{-6}$, FDR < 0.001) and 11 ($P = 1.816 \times 10^{-5}$, FDR = 0.090) shRNAs of the 54,020 shRNAs in the screen. We confirmed ARID1B as a vulnerability by knocking it down in two cell lines that contained ARID1A-inactivating mutations, OVISE and TOV21G, and two ARID1A–wild-type lines, ES-2 and 293T (Fig. 1c and Supplementary Fig. 1b). Proliferation (Fig. 1d) and colony formation (Fig. 1e) were impaired in ARID1A-mutant cells but not in wild-type cells.

ARID1B and ARID1A are 60% identical, have been reported to have opposing functions in cell cycle arrest and are mutually exclusive since individual SWI/SNF chromatin remodeling complexes can contain either ARID1A or ARID1B but not both3. To investigate the relationship between ARID1A and ARID1B in cancer, we asked whether an ARID1B-containing SWI/SNF complex was present in ARID1A-mutant cells. Immunoprecipitation of the SMARC1 (also known as BAF155) core subunit of the SWI/SNF complex resulted in co-precipitation of ARID1B and other SWI/SNF subunits in both wild-type and ARID1A-mutant cells, indicating that intact ARID1B-containing complexes are present (Supplementary Fig. 2a,b) in both wild-type and ARID1A-mutant cells.

We next sought to determine whether ARID1B loss affects the composition of the SWI/SNF complex in ARID1A-mutant cancer cells. Knockdown of ARID1B in wild-type cell lines had no effect on the expression of other SWI/SNF complex subunits or on their incorporation into the complex (Fig. 2a and Supplementary Fig. 2c). However, depletion of ARID1B in ARID1A-mutant cells resulted in dissociation of the core catalytic ATPase subunit SMARCA4 (also known as BRG1) and reduced incorporation of several other subunits (Fig. 2a). Protein levels of core subunits such as SMARCA4, SMARC2 and SMARCB1 were also lowered by ARID1B knockdown in ARID1A-mutant cells, particularly in the TOV21G line (Fig. 2a), whereas the mRNA levels were largely unaffected (Supplementary Fig. 3), suggesting post-translational loss of these proteins.

To further investigate how ARID1B loss affects assembly of the SWI/SNF complex, we performed a sucrose sedimentation assay on cells treated with either control shRNA or ARID1B shRNA. Consistent with the co-immunoprecipitation results, we observed an intact 2-MDa SWI/SNF complex in ARID1A-mutant cells treated with control shRNA (Fig. 2b, full blots in Supplementary Figs. 4 and 5) and in wild-type cells treated with either control or ARID1B shRNA (Supplementary Fig. 6). In contrast, knockdown

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of ARID1B in ARID1A-mutant cells eliminated the intact SWI/SNF complex (Fig. 2b; additional subunits shown in Supplementary Figs. 4 and 5), indicating that in human ARID1A-mutant cancer cell lines, the presence of ARID1B is essential for formation or stabilization of an intact SWI/SNF complex. Previously, the PBRM1 (also known as BAF180) subunit was reported to exist solely in the PBAF subtype of SWI/SNF complexes, whereas both ARID1A and ARID1B were reported to be restricted to the BAF subtype. Our findings demonstrate that PBRM1 association with smaller complexes was substantially affected by the combined loss of ARID1A and ARID1B (Supplementary Figs. 4 and 5), which is consistent with a more recent publication that found that these subunits can coexist⁴. As the SWI/SNF complex binds up to one-third of all genes⁵ and several members of the SWI/SNF complex are essential in mouse development⁶–⁸ and for survival of many cell lineages⁹, loss of an intact SWI/SNF complex would be predicted to be incompatible with cell viability.

In order to further validate the identification of ARID1B as a vulnerability in ARID1A-mutant human cancer, we sought to investigate whether inactivation of Arid1a creates a dependence upon Arid1b using primary mouse embryonic fibroblasts (MEFs) with conditional deletion of Arid1aⁱ⁰. Deletion of Arid1a or knockdown of Arid1b individually had only moderate effects on proliferation, whereas combined loss led to substantial impairment (Supplementary Fig. 7a). We similarly observed that loss of Arid1a or Arid1b alone had only modest effects on the composition of the complex (Fig. 2c), whereas loss of both led to dissociation and degradation of Smarca4 and substantial reductions in the stability and incorporation of many other SWI/SNF subunits (Fig. 2d). Finally, a sucrose sedimentation assay showed that loss of Arid1a and Arid1b in MEFs eliminated the intact SWI/SNF complex (Fig. 2d and Supplementary Fig. 7b).

Collectively, these findings demonstrate a synthetic lethal relationship between this mutually exclusive pair of SWI/SNF subunits. Notably, however, ARID1B has also been reported mutated in human cancers³,¹¹ and has been found to be mutant in some of the same types of cancer as ARID1A, such as neuroblastoma⁴,⁵. As we found ARID1B knockdown to impair the growth of ARID1A-mutant cancer cell lines, we initially hypothesized that mutations in ARID1A and ARID1B would be mutually exclusive. Surprisingly, we found that ARID1A and ARID1B mutations co-occur in both cancer cell lines and primary tumors. Using data from cell line sequencing ¹², we found that 38% of 34 ARID1A-mutant lines also contained ARID1B-inactivating mutations (Supplementary Table 1, P < 1 × 10⁻⁶). Notably, all lines retained at least one allele of either ARID1A or ARID1B, suggesting that retention of at least one ARID1 allele may be essential for survival. This finding also held true in primary cancer samples. We found that of the 297 ARID1A-mutant primary cancer samples cataloged in the cBio Portal for Cancer Genomics¹³, 30 (10.1%) also contained ARID1B mutations (P = 1.07 × 10⁻⁷), which is a significantly higher rate than the 3% rate in ARID1A-wild-type tumors.

The co-occurrence of ARID1A and ARID1B mutations raises the possibility that the synthetic lethality relationship could be caused simply by the high frequency of ARID1B mutations in ARID1A-mutant cancer cell lines. To evaluate this possibility, we removed all ARID1B-mutant cell lines from our analysis and conducted a revised class comparison in which four ARID1B–wild-type, ARID1A-mutant cell lines were compared to 49 cell lines wild type for both ARID1A and ARID1B. ARID1B still scored number 4 out of over 9,000 genes
Figure 2 ARID1B is required for the maintenance of an intact SWI/SNF complex in ARID1A-mutant cancer cell lines and primary cells. (a) Immunoprecipitation (IP) of the SWI/SNF complex by SMARCC1 from the nuclear extracts of ES-2, OVISE and TOV21G cells upon treatment with control shRNA or two independent ARID1B shRNAs and immunoblotted for SWI/SNF complex subunits ARID1A, ARID1B, SMARCA4, PBRM1, SMARCC1, SMARCC2, SMARCD1, SMARCE1, ACTL6A and SMARCBL1. ACTIN is a loading control. (b) Sucrose sedimentation (20–50%) assay of SWI/SNF complex from ARID1A-mutant TOV21G cells treated with either control shRNA (top half) or ARID1B shRNA (bottom half) and immunoblotted for the indicated SWI/SNF complex subunits. (c) Immunoprecipitation of the SWI/SNF complex by SMARCC1 from the nuclear extract of MEFs with indicated treatment and blotted for the indicated SWI/SNF complex subunits. (d) Sucrose sedimentation (20–50%) assay of SWI/SNF complex from the nuclear extract of MEFs with indicated treatment: control shRNA treated MEFs (top half) or Arid1a knockout (KO) and Arid1b knockdown (KD) MEFs (bottom half) and blotted for the indicated SWI/SNF complex subunits. (e) Model: Inactivating mutations in ARID1A promote oncogenic transformation but also create specific dependency on ARID1B. Inhibition of ARID1B in ARID1A-mutant cells destabilizes the SWI/SNF complex and results in impaired cell growth.

(P = 7.154 × 10^{-4}), indicating that the synthetic lethality between ARID1A and ARID1B is a result of ARID1A mutation and not co-occurring ARID1B mutations.

In this report, we show that inactivating mutations in ARID1A, frequent across a wide variety of cancers, create a dependency upon ARID1B (Fig. 2e). It is notable that the number 1 vulnerability in ARID1A-mutant cell lines is another member of the SWI/SNF complex. We previously showed that cancer formation in the absence of the SWI/SNF subunit SMARCBL1 does not result from SWI/SNF inactivation but rather is dependent upon the activity of the residual SWI/SNF complex. At that time we speculated that, much like in oncogene addiction, cells carrying SMARCBL1 mutations may be ‘addicted’ to an aberrant residual SWI/SNF complex and that targeting this complex might theoretically be an effective therapeutic approach for SMARCBL1-mutant cancers. Our present study reveals the role of the residual complex in the growth of ARID1A-deficient cancers and also identifies a specific subunit as a relative vulnerability. This principle may have broad applicability to SWI/SNF-mutant cancers, similar to the case Oike et al. recently showed in which SMARCA2, a paralog of SMARCA4, was essential in SMARCA4-mutant cancers.

Together, our findings may suggest that partial loss of ARID1 function via mutation of ARID1A alleles or, less frequently, ARID1B alleles, can drive cancer growth but at the same time create a specific vulnerability compared to nonmutant cells. This suggests ARID1B as a potential therapeutic target for cancers that contain inactivating ARID1A mutations. Recent examples have demonstrated the feasibility and efficacy of targeting chromatin regulators such as BRD4 (ref. 16) as well as other non-enzymatic proteins such as BCL-2 (ref. 17) and molecules previously found difficult to target, such as RAS18. ARID1B could potentially be targetable through its E3 ubiquitin ligase interaction19. Additionally, new approaches using small stabilized peptides could potentially be targetable through its E3 ubiquitin ligase interaction.

Methods
Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.
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AUTHOR CONTRIBUTIONS

C.W.M.R. directed the study. K.C.H. and X.W. designed and performed experiments. J.R.H. and H.E.M. performed experiments. C.W.M.R., K.C.H., X.W., B.G.W., J.R.H., H.E.M. and Z.J. analyzed and interpreted the data. K.C.H., B.G.W., F.V. and A.J.A. analyzed Project Achilles data. G.V.K., M.G. and L.A.G. provided and analyzed sequencing data. Z.W. provided Arelia-conditional mice, intellectual contribution and useful discussion. Y.K. established the Arelia-conditional mouse strain. W.C.H. directs the Achilles Project, provided reagents, helped interpret Achilles data and edited the manuscript. C.W.M.R., K.C.H. and X.W. wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.
ONLINE METHODS

Achilles analysis. To find genes that are preferentially essential in mutant cell lines, we used the GenePattern module PARIS (http://www.broadinstitute.org/cancer/software/genepattern/) using the default parameters except quality, which was changed to final. The gene-level Achilles data set v2.4 was used as a data file (http://www.broadinstitute.org/achilles/) (file name: Achilles_QC_v2.4_rnal.Gs.gct; see also Supplementary Data). The classifier files were generated using the gene mutation status from the Cancer Cell Line Encyclopedia (http://www.broadinstitute.org/ccle). Cell lines without hybrid capture sequencing data were removed from the analysis. The mutational status of ARID1A was annotated for 165 of the 216 cell lines in the Achilles data set, and, as a result, these 165 cell lines were used in the class comparisons.

Cell culture. TOV21G (CRL 11730), ES-2 (CRL-1987) and 293T (CRL-3216) cell lines were purchased from American Type Culture Collection. OVISE cells were obtained from W.H.C.'s laboratory. Mouse embryonic fibroblasts (MEFs) were generated as described previously. Cells were transduced with shRNAs and selected with puromycin for 48–72 h before seeding for MTT or colony formation assays. MTT assays were conducted with a Cell Proliferation Kit (Roche). Colony formation assays were conducted by staining cells for 20 min with crystal violet staining solution (0.05% crystal violet, 1% formaldehyde, 1% PBS, 1% methanol).

shRNA-mediated knockdown of ARID1B. ARID1B shRNAs were obtained from the RNA interference (RNAi) screening facility at the Dana-Farber Cancer Institute and were lentivirally transduced into OVISE, TOV21G, ES-2 and 293T cells. ARID1B and nonsilencing control shRNAs are in the pLKO.1 lentiviral expression vector backbone. Target sequences for shRNAs are available upon request.

Density sedimentation analysis. Nuclear extract (1 mg) was diluted in 300 µl of 0% sucrose RIPA buffer and carefully overlaid onto a 12-ml 20–50% sucrose gradient prepared in a 14 ml 14 × 95 mm polycrylamide centrifuge tube (Beckman Coulter, cat. # 331374). Tubes were placed in an SW-40 Ti swing bucket rotor and centrifuged at 4 °C for 16 h at 40,000 r.p.m. Fractions (0.5 ml) were collected and used in gel electrophoresis and subsequent western blotting analyses.

Immunoblots and co-immunoprecipitation experiments. Whole-cell extracts for immunoblotting were prepared by incubating cells on ice in 1% NP-40 lysis buffer (50 mM Tris-HCl pH 7.4, 5 mM EDTA, 12% glycerol, 50 mM NaCl, 1% NP-40) plus protease inhibitors (Complete, Mini, EDTA-free. Roche: 1183617001) for 30 min. Supernatants were collected following a brief spin (10 min) at 17,900 r.c.f. to separate cellular debris in a 4 °C centrifuge. Protein concentrations were determined using the Bradford reagent (Bio-Rad). SDS-PAGE was used to separate proteins, which were subsequently transferred to polyvinylidene difluoride membranes (Millipore). ARID1B-specific antibody (Abcam: ab54761, 1:1,000) was used to detect efficient knockdown.

Nuclear extracts for immunoprecipitation were prepared using the NE-PER Nuclear and Cytoplasmic Extraction Kit (Thermo Scientific: 78833). Nuclear extracts were diluted with RIPA buffer (1 mg/ml, with protease inhibitors and DTT). Each IP was incubated with antibodies overnight at 4 °C. Protein G Dynabeads (Life Technologies: 10004D) were added and incubated at 4 °C for 3 h. Beads were then washed three times with RIPA buffer and resuspended in reducing SDS gel loading buffer. Antibodies to the following proteins were used in the immunoprecipitation and immunoblots: SMARCC1/BAF155 (Santa Cruz: sc7946, 1:1,000); ARID1A (Bethyl Laboratories: A301-041A, 1:1,000); PRDM1 (Bethyl Laboratories: A301-591A, 1:3,000); SMARCA4 (Santa Cruz: sc17796, 1:500); SMARCC2/BAF170 (Bethyl Laboratories: A301-039A, 1:3,000); SMARCD1/BAF60A (Bethyl Laboratories: A301-595A, 1:3,000); SMARCE1/BAF57 (Bethyl Laboratories: A300-810A, 1:3,000); ACTL6A/BAF53A (Bethyl Laboratories: A301-391A, 1:3,000); ACTIN (Cell Signaling Technology: 5125, 1:3,000).

RNA purification and RT-qPCR. Total RNA was extracted using Trizol reagent (Invitrogen) following the manufacturer’s instructions. 2 µg of total RNA was reverse-transcribed into first-strand cDNA using oligo(dT)20 primers and the SuperScript III Reverse Transcriptase (Invitrogen). RT-qPCR was performed on the ViiA 7 Real-Time PCR System (Life Technologies) using SYBR Select Master Mix (Life Technologies). Reactions were performed in triplicate, and gene expression was normalized to GAPDH. Error bars represent s.d. of mean expression.

Cell line sequencing. Cell line genomes were sequenced as previously described. Cell line sequencing data and the data from the Cancer Cell Line Encyclopedia were used to identify cell lines with co-occurring mutations of ARID1A and ARID1B.

Statistical significance of mutation overlap. To evaluate the statistical significance of the overlap of ARID1A and ARID1B mutations, the probability of observing cell lines with both mutations was estimated under the null hypothesis that these two mutations are independent. For that, n1 represents cell lines with ARID1A mutation, n2 represents cell lines with ARID1B mutation and n12 represents cell lines with both mutations. The following simulation was run: n1 cell lines were randomly picked with the probability for each cell line being selected set relative to its mutation rate and assigned mutation ‘A’ to these cell lines. Next, n2 cell lines were similarly selected and assigned mutation ‘B’ to those cell lines, and then the number of cell lines with both mutations ‘A’ and ‘B’ was counted. This process was repeated many times to estimate the probability of observing n12 cell lines or more with both mutations.

For primary cancer samples, a contingency table was formed consisting of the counts for all the four possibilities of ARID1A or ARID1B mutation status. The Fisher’s exact test was used to calculate the statistical significance of the overlap of ARID1A and ARID1B mutations.

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