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ARID1A, a SWI/SNF subunit, is critical to acinar cell homeostasis and regeneration and is a barrier to transformation and epithelial-mesenchymal transition in the pancreas.

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Abstract

Objective—Here, we evaluate the contribution of AT-rich interaction domain-containing protein 1A (*ARID1A*), the most frequently mutated member of the SWItch/Sucrose Non-Fermentable (SWI/SNF) complex, in pancreatic homeostasis and pancreatic ductal adenocarcinoma (PDAC) pathogenesis using mouse models.

Design—Mice with a targeted deletion of *Arid1a* in the pancreas by itself and in the context of two common genetic alterations in PDAC, *Kras* and *p53*, were followed longitudinally. Pancreases were examined and analysed for proliferation, response to injury, and tumourigenesis. Cancer cell lines derived from these models were analysed for clonogenic, migratory, invasive and transcriptomic changes.

Results—*Arid1a* deletion in the pancreas results in progressive acinar-to-ductal metaplasia (ADM), loss of acinar mass, diminished acinar regeneration in response to injury, and ductal cell

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expansion. Mutant *Kras* cooperates with homozygous deletion of *Arid1a* leading to intraductal papillary mucinous neoplasm (IPMN). *Arid1a* loss in the context of mutant *Kras* and *p53* leads to shorter tumour latency, with the resulting tumours being poorly differentiated. Cancer cell lines derived from *Arid1a*-mutant tumours are more mesenchymal, migratory, invasive and capable of anchorage-independent growth; gene expression analysis showed activation of epithelial-mesenchymal transition (EMT) and stem cell identity pathways that are partially dependent on *Arid1a* loss for dysregulation.

Conclusions—ARID1A plays a key role in pancreatic acinar homeostasis and response to injury. Furthermore, ARID1A restrains oncogenic KRAS-driven formation of premalignant proliferative IPMN. *Arid1a*-deficient PDAC are poorly differentiated and have mesenchymal features conferring migratory/invasive and stem-like properties.

Keywords

Pancreatic Ductal Adenocarcinoma; ARID1A; SWI/SNF; IPMN; EMT

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is often diagnosed at an advanced stage that precludes surgery and is largely resistant to chemotherapy.¹ There is a need for improved therapies based on the molecular mechanisms underlying PDAC.² Common genetic alterations in PDAC include *KRAS*, *TP53*, *SMAD4/DPC4*, and *CDKN2A/p16INK4A*,³⁻⁷ as well as in the chromatin remodeling SWI/SNF complex, with *ARID1A* being the most frequently altered.⁸⁻¹⁰ The human SWI/SNF complex exists in various combinatorial assemblies that include an ATPase, “core” elements, and other subunits including ARID1A that confer functional specificity.¹¹ SWI/SNF is known to be involved in transcriptional regulation, DNA replication and damage repair,¹²⁻¹⁴ with *ARID1A*, in particular, implicated in regulation of development and cell-cycle control frequently through opposition of polycomb-group (PcG) proteins such as EZH2.¹⁵⁻¹⁸ Mutations in *ARID1A* render certain cancers susceptible to particular pharmacologic interventions including EZH2, PI3K, AKT, ATR, PARP, and HDAC6 inhibitors,^{14,19,20} providing potential new therapeutic options.

The SWI/SNF complex appears broadly to control malignant progression and the differentiation states of tumours. BRG1 (SMARCA4), an ATPase component of the SWI/SNF complex, is needed for terminal maturation of pancreatic ducts and its loss cooperates with oncogenic KRAS in pancreatic ductal cells to form intraductal papillary mucinous neoplasm (IPMN).²¹ BRG1 re-expression induces a mesenchymal phenotype in established PDAC cell lines that have developed in its absence.²² SMARCB1, via regulation of MYC, may restrain the expansion of mesenchymal subpopulations associated with de-differentiation and aggressive behaviour.²³ ARID1B exhibits tumour suppressor activities when re-expressed in the human MiaPaCa2 PDAC cell line where it is repressed by promoter hypermethylation.²⁴ In other cancers, ARID1A alters pathways related to cell-cell adhesion and invasion.^{13,25-27}

We evaluated the impact of *Arid1a* loss in the pancreas using mice with conditional mutations of *Arid1a*, as well as the commonly occurring PDAC mutations *Kras* and *p53*. Our

results show that ARID1A is critical to the maintenance of pancreatic acini and constrains proliferation of the pancreatic ductal compartment. *Arid1a* loss shifted the spectrum of cancer precursors from PanIN to IPMN and accelerated the onset of invasive cancer. Cell lines derived from *Arid1a*-mutant PDAC tumours demonstrated increased migration, invasion and anchorage-independent growth supported by an EMT and stem-like transcriptional program. Re-establishing *Arid1a* expression and inhibition of PDGFR β with imatinib both partially abrogate this phenotype, offering a potential path to mutation-directed therapy.

RESULTS

Loss of ARID1A leads to progressive pancreatic atrophy and cysts

Given the importance of ARID1A in developmental processes, tissue homeostasis, and regeneration,¹⁶¹⁷²⁸ we generated cohorts of mice carrying *Ptf1a*-Cre (*C*), *Arid1a*^{*fllox/fllox*}(*A*), *Ptf1a*-Cre; *Arid1a*^{*fllox/f+*} (*CA*^{*f/+*}) or *Ptf1a*-Cre; *Arid1a*^{*fllox/fllox*} (*CA*^{*f/f*}) alleles (supplementary figure S1A,B). *Ptf1a* is expressed in multipotent progenitor cells, which give rise to the three main cellular lineages in the pancreas.²⁹ The *Arid1a*^{*fllox*} allele enables deletion of the eighth exon of *Arid1a* in the presence of Cre recombinase leading to frameshift mutation and nonsense-mediated mRNA decay.¹⁷ *CA*^{*f/f*} mice were generated at expected Mendelian ratios, developed normally, and survived through a year of age. *CA*^{*f/f*} pancreases (figure 1D,H,L) showed progressive loss of acinar cell mass compared with all other controls (figure 1A-C,E-G,I-K), which were grossly and histologically normal. Histologic analysis at 12 weeks showed apoptosis, acinar-to-ductal metaplasia (ADM, n=12/12), focal pancreatitis (11/12), and microscopic cysts (9/12) (figure 1H and supplementary table S1) and were smaller than controls (supplementary figure S1H). With age, *CA*^{*f/f*} demonstrated ongoing ADM, acinar loss, fat accumulation consistent with pancreatic atrophy, and dilatation of a simple columnar ductal epithelium into grossly visible fluid-filled cysts (figure 1D-white arrow,L and supplementary figure S1C-G). Immunohistochemistry (IHC) confirmed ARID1A absence in *CA*^{*f/f*} acinar and ductal cells (figure 1M,N). Retained expression in islets suggested that islet development may depend on ARID1A and is consistent with developmental outgrowth of islets arising from non-*Ptf1a*-expressing progenitors.³⁰³¹ Thus, we find *Arid1a* mutation leads to progressive loss of the acinar cell compartment, ADM, and progression of ductal cystic lesions.

ARID1A is required for acinar cell recovery and suppresses ductal proliferation.

As ARID1A facilitates differentiation, maintenance of epithelial populations, and recovery after injury,¹⁶¹⁷²⁸ we evaluated the effect of injury on *CA*^{*f/f*}, *C* and *A*^{*f/f*} cohorts by injection of caerulein or vehicle control at 8 weeks of age twice daily for two weeks (supplementary figure S2A).³² *CA*^{*f/f*}, *C*, and *A*^{*f/f*} were equally susceptible to injury, loss of acini, and ADM (figure 2A1&2,B1&2,G 0 days post-injection [dpi] and supplementary figure S2L,N). *CA*^{*f/f*} pancreases were grossly smaller and failed to recover normal acinar cell mass (figure 2D1&2,F1&2,G) compared to controls at 3 and 9 dpi (figure 2C1&2,E1&2 and supplementary figure S2B-N). IHC for PDX1, critical to acinar cell development and identity,³³ demonstrated robust expression in the re-emerging acinar units in caerulein-treated controls that was diminished in *CA*^{*f/f*}, suggesting an inability to activate this program

in the absence of ARID1A (figure 3A-D). Ducts in CA^{ff} pancreases, at baseline and after injury, stained positively for PDX1, compared with controls, consistent with previous reports that *Brg1*-mutant ductal structures have a de-differentiated (PDX1-positive) phenotype.²² Immunofluorescence (IF) for MIST1 (Muscle Intestine and Stomach Expression 1, a marker of terminally differentiating acinar cells),³⁴³⁵ present at baseline in CA^{ff} and controls (figure 3E1-H1,I and supplementary figure S3A,B), and the proliferative marker Ki-67 (figure 3E2-H2) demonstrated suppression of both total MIST1-positive cells (figure 3E1-H1), and co-stained cells (proliferating acinar compartment) among CA^{ff} post-injury compared with controls (figure 3E-I and supplementary figure S3A-D), supporting the observed failure of CA^{ff} to reconstitute acinar cells. IF for the ductal marker Cytokeratin 19 (CK19) and Ki-67 similarly showed decreased proliferation in the CK19-negative (acinar) cells of caerulein-treated CA^{ff} (supplementary figure S3E,F,I). To exclude the possibility of *Arid1a*-mutant ductal structures impacting acinar recovery, we used a duct-specific, tamoxifen-inducible Cre-recombinase allele (*Ck19-CreERT*) to delete *Arid1a* in mature ducts (supplementary figure S4A-I). This had no appreciable impact on post-injury acinar recovery, MIST1 expression, or proliferation (supplementary figure S5A-I), demonstrating that impaired acinar recovery was likely due to cell autonomous effects of *Arid1a* mutation.

ARID1A IHC showed enrichment of ARID1A-expressing cells throughout the post-injury time course among CA^{ff} pancreases as compared with controls (figure 2A3-F3 and supplementary figure S2F,K), suggesting a selective advantage for ARID1A-intact cell populations (cells escaping Cre-mediated ARID1A deletion) during recovery. Consistent with an ARID1A-competent sub-population underlying this limited acinar recovery, IF for ARID1A and Ki-67 showed co-localization in many of the proliferating cells in injured CA^{ff} pancreases (supplementary figure S6A-D). These data further support the conclusion that ARID1A loss leads to a block of physiologic post-injury proliferation and recovery of the acinar lineage with both diminished numbers of cells directed towards an acinar fate as well as a diminished proliferative capacity. Thus, ARID1A is necessary to maintain pancreatic acinar integrity over adult life and reconstitution following insult.

To better understand the basis for the histologic changes in CA^{ff} mice, we performed IHC for Ki-67 and cleaved Caspase-3 (CC3), a marker of apoptosis. At 12 weeks, CA^{ff} pancreases had higher rates of Ki-67 and CC3 positive cells than A^{ff} (supplementary figure S7A-D), signifying increased basal levels of apoptosis and proliferation. We observed that apoptosis occurred among acinar cells, consistent with progressive attrition over time, while the difference in Ki-67 staining was seen in ducts (supplementary figure S7E,F). To specifically evaluate the ductal compartment, we performed IF for CK19 and Ki-67 of CA^{ff} and A^{ff} at 12, 20, and 52 weeks. At each time point, CA^{ff} pancreases showed increased rates of Ki-67 in the CK19-positive compartment that increased over time (figure 4A-G and supplementary figure S3G-I), indicating that ARID1A restrains ductal proliferation.

***Arid1a* mutation causes IPMN in *Kras* and *p53* mutant PDAC models**

The mutational activation of *KRAS* is the most common genetic mutation in PDAC and is an early event in pancreatic tumorigenesis.³⁶³⁷ To determine if *Arid1a* mutation cooperates with oncogenic *Kras*^{G12D}, we utilised the *Lox-stop-lox Kras*^{G12D} (*LSL-Kras*^{G12D}) allele³⁸

that undergoes Cre-mediated excision of an in-frame stop codon leading to expression of constitutively active *Kras*^{G12D} under the endogenous promoter to generate *Ptf1a-Cre; LSL-Kras*^{G12D} (*KC*), *Ptf1a-Cre; LSL-Kras*^{G12D}; *Arid1a*^{f/+} (*KCA*^{f/+}) and *Ptf1a-Cre; LSL-Kras*^{G12D}; *Arid1a*^{f/f} (*KCA*^{f/f}) mice (supplementary figure S1A,B). *KC*, *KCA*^{f/+}, and *KCA*^{f/f} cohorts were followed longitudinally and sacrificed at signs of illness.

KCA^{f/f} mice had distended abdomens beginning at ~20 weeks of age and a diminished overall survival (OS, defined by death due to any cause) compared with *KC* controls (supplementary figure S8A, 48.57 weeks vs. not reached, *p*<0.0001). At necropsy, expansive fluid-filled cystic pancreases were found in all *KCA*^{f/f} mice. Analysis at 52 weeks of age demonstrated that this was not seen among *KC* and variably and less prominent among *KCA*^{f/+} (figure 5A-C and supplementary figure S8B,D). Widespread ductal proliferative lesions with varying degrees of cellular atypia forming large interconnecting cystic structures, morphologically consistent with IPMN, were found in *KCA*^{f/f} pancreases (figure 5F,I). *KCA*^{f/f} cohort harbored invasive, poorly-differentiated cancer with metastasis in 3 of 19 mice. Though most deaths were due to abdominal distention from IPMN, the *KCA*^{f/f} cohort exhibited a greater incidence of invasive cancer with a diminished cancer-specific survival (CSS, defined by death with histologic evidence of cancer) compared with *KC* controls (62.43 weeks vs. not reached, *p*=0.0166, figure 5N, supplementary figure S8I-K and supplementary table S2A,C,D). Higher rates of invasive PDAC were found in the *KCA*^{f/+} cohort (*n*=6 of 15 [40%]) compared with *KC* group (*n*=1 of 11 [9.1%]), thus heterozygosity of *Arid1a* in the setting of *Kras* mutation may also accelerate PDAC formation (figure 5N, supplementary figure S8F-H and supplementary table S2A,B,D). Comparison of the pre-neoplastic lesions in mice sacrificed at earlier time points revealed a shift in the spectrum of precursor lesions; *KCA*^{f/f} harbored more IPMN while *KC* harbored more high-grade, PDX1-positive PanIN (figure 5D-I,M, supplementary figure S8B-E and supplementary table S2A-D) with *KCA*^{f/+} harboring both PanIN lesions and variable cyst formation (figure 5E,H and supplementary table S2B). ARID1A IHC showed intact expression in *KC* (figure 5J), mosaic loss in *KCA*^{f/+} (particularly in the ducts, figure 5K), and total loss in epithelia of IPMN lesions in *KCA*^{f/f} (figure 5L). These data imply that ARID1A plays a tumour suppressive role in the pancreatic epithelia in the context of oncogenic *Kras*^{G12D} and is sufficient to drive expansion of IPMNs and accelerate transformation.

Kras mutation alone targeted to the murine pancreas leads to PDAC with a long latency that is accelerated with concurrent mutation in other cooperating tumour suppressor genes such as *Cdkn2a* or *p53*.³⁴⁷ To determine the impact of *Arid1a* mutation in the context of a highly penetrant and fully invasive cancer model, we utilised the *p53*^{fllox/+} (*p53*^{f/+}) allele that undergoes Cre-mediated excision of exons 2 through 10 rendering the gene functionally inactive.³⁹ We generated mice carrying *Ptf1a-Cre; LSL-Kras*^{G12D}; *p53*^{f/+}; *Arid1a*^{f/+} (*KPCA*^{f/+}) as well as *Ptf1a-Cre; LSL-Kras*^{G12D}; *p53*^{f/+}; *Arid1a*^{f/f} (*KPCA*^{f/f}) and compared them to littermate control *Ptf1a-Cre; LSL-Kras*^{G12D}; *p53*^{f/+} (*KPC*) animals (supplementary figure S1A,B).

Survival analyses did not reveal any differences in OS between *KPC*, *KPCA*^{f/+}, and *KPCA*^{f/f} cohorts (figure 6N). *KPCA*^{f/f} mice had large, cystic pancreases (figure 6C), similar to *KCA*^{f/f}, often with focal nodularity harboring invasive cancer, while *KPC* and *KPCA*^{f/+}

harbored firm, diseased pancreases with invasive cancer (figure 6A,B) absent of extensive cystic changes. *KPCA^{ff}* mice had inferior CSS compared with controls (26.71 vs. 28.00 weeks, $p=0.048$, supplementary figure S9A) and *Arid1a* loss shifted the histologic spectrum of precursors from low- and high-grade PanINs, the dominant lesions found in *KPC*, to predominantly low- and high-grade IPMNs in *KPCA^{ff}* cohorts (figure 6D,F,M and supplementary figure S9B-E), with both PanINs and IPMNs in *KPCA^{ff/+}* (figure 6E,M). PDAC in *KPCA^{ff}* was associated with large IPMN-like cystic lesions (similar to *KCA^{ff}*) with varying grades of dysplasia and cancer (figure 6F,I and supplementary figure S9D). ARID1A IHC confirmed retained expression in *KPC* and *KPCA^{ff/+}* but was lost in *KPCA^{ff}* (figure 6J-L). The majority of *KPCA^{ff}* PDAC ($n=12/13$ [92.3%]) were poorly differentiated compared to *KPC* ($n=18/30$ [60.0%]) or *KPCA^{ff/+}* PDAC ($n=6/13$ [46.2%]) (figure 6G-I and supplementary table S3A-D), but with similarly aggressive metastatic rates (supplementary figure S9F-K and supplementary table S3D). These results indicate that in the setting of *Kras* and *p53* mutation, ARID1A loss leads to IPMN and the development of fully-invasive poorly-differentiated adenocarcinoma with a small decrease in CSS.

As compared with human pathology, *KCA^{ff}* and *KPCA^{ff}* IPMN lesions were found to most closely resemble the gastric subtype, predominantly lacking expression of MUC1 and MUC2 (supplementary figure S10A-C, I-K), with discrete areas of high-grade dysplasia within IPMNs demonstrating a transition resembling either intestinal-type IPMN (MUC2 positive, supplementary figure S10F,G,L-N,R) or pancreatobiliary-type IPMN (MUC1 positive, supplementary figure S10E,O-R), which is consistent with progression of human IPMNs.^{40,41} Furthermore, the epithelia expressed PDX1 (marker of ducts with a progenitor-like phenotype, supplementary figure S8E,S9E) and did not show nuclear expression of ER α in the stroma around the ducts (supplementary figure S10D,H), indicating that these lesions are not consistent with mucinous cystic neoplasms (MCNs).

***Arid1a*-mutant PDAC cell lines are more mesenchymal and invasive**

Primary cell lines derived from *KPCA^{ff}* tumours using established methods²¹ were morphologically more mesenchymal compared to *KPC* lines, which retained an epithelial appearance, corresponding to histologic analysis of the original tumours (figure 7A,B and supplementary figure S11A,B). Somatic mutation of *Arid1a* in *KPCA^{ff}* cancer lines was shown by PCR (supplementary figure S11C) and confirmed by western blot analysis (figure 7C). Cisplatin failed to induce p53 in all *KPC* and all but one *KPCA^{ff}* lines, demonstrating an impaired p53 axis (figure 7C), presumably due to spontaneous loss of heterozygosity (supplementary figure S11C). Comparison of *KPCA^{ff}* with *KPC* lines ($n=2$ per genotype) showed that *Arid1a*-mutant lines exhibited enhanced anchorage-independent growth in a clonogenic assay (figure 7D-F and supplementary figure S11E,F) and faster closure in a wound healing assay (figure 7G-I and supplementary figure S11G-L), consistent with increased migratory potential, while *KPC* lines generally had higher proliferation rates (supplementary figure S11D). Evaluation of the boundaries of colonies revealed extension and invasion of *KPCA^{ff}* cells into the agar, a feature notably absent among *KPC* lines (figure 7D,E insets). Mirroring the more aggressive development of cancer in *KPCA^{ff}* mice, these results were suggestive of ARID1A's role in suppressing cell migration and anchorage-independent growth.

Global transcriptome analyses comparing *KPC* and *KPCA^{ff}* cell lines (n=5 independent lines for each genotype, supplementary table S4) identified 1109 differentially expressed genes (846 upregulated genes and 263 downregulated genes) with p-adj <0.05 (supplementary figure S12A,B). Sample-to-sample distance plot and principal components analysis demonstrated clustering of *KPC* lines separate from *KPCA^{ff}* lines (supplementary figure S12C,D). Gene Set Enrichment Analysis (GSEA) identified “SUZ12 targets” as a significantly enriched pathway (figure 8C) and analyses on the Enrichr platform identified both SUZ12 and EZH2 as transcriptional regulators of the differentially expressed genes (supplementary figure S12E), consistent with other studies that show SWI/SNF complexes oppose epigenetic silencing by polycomb repressive complex 2 (PRC2).¹⁸ Additionally, gene signatures for EMT and stem cells were enriched in *KPCA^{ff}* lines (figure 8A,B,D), consistent with above phenotypic observations. Other gene signatures associated with *KPCA^{ff}* cell lines include invasiveness, alterations to cell-matrix adhesions, and differentiation (supplementary table S5).

Differential expression of genes affecting EMT and metastasis including *Lox* (log2FC 8.33, p-adj 2.55×10^{-38}), *Prrx1* (log2FC 7.43, p-adj 3.96×10^{-38}), and *Fbn1* (log2FC 7.18, p-adj 8.30×10^{-25}) was validated using quantitative polymerase chain reaction (qPCR) in three each of the original cell lines used in RNA-Seq and additionally derived cell lines from *KPC* (n=3 new) and *KPCA^{ff}* (n=5 new) tumours (figure 8E and supplementary table S4). *Pdgfrb* (log2FC 8.63, p-adj 4.00×10^{-31}) and *Fbn1* (log2FC 7.18, p-adj 8.30×10^{-25}) did not demonstrate a significant expression difference between *KPC* and *KPCA^{ff}*, likely due to heterogeneity within the group, although the majority of lines were consistent with the RNA-seq results (supplementary figure S13A,B). Western blot analysis for E-Cadherin showed loss of expression in 3 of 6 *KPCA^{ff}* cell lines as compared to intact expression in all *KPC* cell lines (figure 8F), correlating with a more invasive phenotype.⁴² These results suggest that *KPCA^{ff}* cell lines are less differentiated with increased migratory and invasive potential compared to *KPC* cell lines.

To assess whether ARID1A directly regulates the migration of derivative cancer lines as well as the genes identified by RNA-Seq, *Arid1a* was re-expressed in three of the *Arid1a*-mutant derived cancer lines described above (*KPCA^{ff}* lines 1,6,7) using a Sleeping Beauty transposon system.⁴³ ARID1A re-expression was confirmed by qPCR and Western blot (figure 9A and supplementary figure S14B). Additionally, qPCR confirmed that re-expression decreased mRNA levels of *Pdgfrb*, *Lox*, and *Lef1* in two of three cell lines tested for each gene though not of all genes tested (figure 9A and supplementary figure S14A), suggesting a direct role of ARID1A in persistent regulation of some of these metastasis-associated genes. Furthermore, ARID1A reexpression led to diminished migration using the wound healing assay, demonstrating that its mutation is important to maintaining this migratory phenotype (figure 9B).

Pdgfrb, among the top genes found differentially expressed between *KPC* and *KPCA^{ff}* cell lines (figure 8D), is a known driver of invasion, migration, and metastasis.⁴⁴ To assess whether pharmacologic inhibition of this actionable target could impact these behaviors, we treated cell 3 *KPC* and 3 *KPCA^{ff}* lines with inhibitors of PDGFRb (imatinib). Imatinib (1 μ M) significantly inhibited the migratory (2 of 3 lines, figure 9C and supplementary figure

S15F-M) and invasive (3 of 3 lines, figure 9D and supplementary figure S16M-R) abilities of *KPCA^{f/f}* lines, while having no impact on proliferative (supplementary figure S15A), migratory (figure 9C and supplementary figure S15B-E), clonogenic or invasive characteristics of *KPC* lines compared to *KPCA^{f/f}* (supplementary figure S16A-R). These data suggest that dysregulation of PDGFRb downstream of *Arid1a* loss is partly responsible for the invasive characteristics of *KPCA^{f/f}* cell lines and potentially targetable with existing drugs. Interestingly, the cell line whose migratory capacity was unaffected by *Arid1a* re-expression was the same cell line in which *Arid1a* re-expression did not alter *Pdgfrb* expression yet was still sensitive to imatinib treatment (cell line 7, figure 9A-C). In summary, *KPCA^{f/f}* cell lines which are migratory and enriched in gene signatures associated with PRC2, EMT, and stem-like phenotypes can be rendered less migratory with *Arid1a* re-expression or with targeting of the PDGFRb axis with imatinib.

DISCUSSION

We report here the characterisation of a pancreas-specific conditional knockout of *Arid1a* by itself and in the context of other known driver mutations, *Kras* and *p53*. *Arid1a* is critical to maintain acinar cell mass through the adult life of the organism and for the normal function of an acinar re-differentiation program following injury, while serving in the ductal compartment to restrain proliferation. In pancreatic cancer models, mutation of *Arid1a* leads to IPMN precursors and the acceleration of cancer formation with a more migratory and mesenchymal phenotype supported by transcriptomic programs consistent with unopposed PRC2 function as well as activated EMT and stem cell pathways. These phenotypes and select downstream targets including PDGFRb are dependent on ARID1A loss for de-regulation and are potentially targetable.

Pancreatic acinar cells are highly differentiated and specialised, functioning to produce digestive enzymes important for the breakdown of nutrients in the gut. ARID1A has been found to be important for achieving or maintaining states of differentiation in hepatocytes and cardiomyocytes¹⁶¹⁷²⁸ and similarly we find here that ARID1A is necessary for supporting acinar cell function and repopulation following injury with altered expression of key transcription factors required for acinar fate specification, PDX1 and MIST1.³³³⁴ The importance of ARID1A in maintaining enhancer accessibility¹³ and the recent identification of a transcriptional enhancer critical to *Mist1* expression³⁵ raises the possibility of *Arid1a* mutation leading to dysfunction at this critical locus. In contrast to the phenotype in acinar cells, we observed expansion and proliferation of a PDX1-expressing ductal compartment that is amplified with progressive age and consistent with a progenitor-like state within the ducts. This proliferative phenotype may be caused by dysregulation of cell-cycle checkpoints,¹⁵ failure to achieve or maintain terminal differentiation, as seen with loss of BRG1²¹ or suppression of senescence.⁴⁵⁻⁴⁷

The shift of pancreatic precursors, from low- through high-grade PanINs to IPMNs and low-grade PanINs with homozygous deletion of *Arid1a* in the context of *Kras^{G12D}* with or without hemizygous deletion of *p53* raises the possibility that *Arid1a* is required for the transition to high-grade PanIN. Consistent with a recent report,⁴⁸ we show that *CA^{f/f}* mice develop macroscopic proliferative cysts, wherein addition of oncogenic KRAS gives rise to

IPMNs, suggesting ARID1A is a barrier to ductal expansion. Studies showing that acinar cells give rise to PanIN⁵⁴⁹⁵⁰ while ductal cells give rise to IPMN using compartment-specific Cre recombinases are in keeping with this.²¹ However, PanINs have been reported to develop in *KCA^{f/f}* mouse pancreases,⁴⁸ and indeed we did see PanINs in *KCA^{f/f}*, but no examples of high-grade PanINs, whereas similarly-aged *KC* mice did show these lesions, suggesting the possibility of bystander PanINs in cells escaping the effect of Cre⁵¹ or different biological outcomes of *Arid1a* deletion in embryologic as opposed to adult pancreases. Thus, ARID1A may play a dichotomous role in each epithelial compartment, inhibiting IPMN progression while potentially being necessary for the progression to high grade PanIN, consistent with data on the effect of *Brg1* and other chromatin modulators in the pancreas²¹²²⁵² and pleomorphic phenotypic outcomes observed in the liver.⁴³

Concomitant with the shift to IPMN, we observed a decreased latency in cancer-related death, demonstrating that *Arid1a* plays a potent tumour suppressive role in the development of PDAC from IPMNs consistent with its observed loss in human disease. The IPMNs in *KCA^{f/f}* and *KPCA^{f/f}* cohorts mostly resembled the gastric subtype, but some areas of higher-grade pathology had characteristics resembling intestinal and pancreatobiliary subtypes, consistent with transitions in phenotype seen in human disease.⁴⁰⁴¹⁴⁸ Taken together, our data suggest that in the presence of oncogenic KRAS, biallelic loss of *Arid1a* drives the expansion and transformation of the ductal compartment.

Arid1a-deficient PDAC are poorly differentiated and derivative cell lines have a greater capacity for migration, invasion, anchorage-independent growth, and an EMT as well as stem-like transcriptomic profile. Poorly differentiated tumours have been found to be associated with overexpression of genes enriched in embryonic stem cells⁵³ and other models of cancer with loss of function of SWI/SNF components exhibit similar characteristics.¹³ Beyond genetic lesions, down-regulated expression of SWI/SNF components are known to underlie states of mesenchymal differentiation and reprogramming.²³ Loss of these complexes can promote differentiation in breast and ovarian cancers,²⁷⁵⁴ however, consistent with our data, other gastrointestinal cancers become more mesenchymal and/or have poorer prognosis in the absence of ARID1A.²⁵²⁶⁴³⁵⁵ ARID1A has been shown to target the SWI/SNF complex to enhancers in a colon model¹³ and SMARCB1 loss has been shown to alter binding to typical enhancers with redistribution of SWI/SNF complexes that may impair SMARCB1-deficient cells to differentiate while maintaining other features that promote tumourigenesis, such as self-renewal, survival and proliferation.⁵⁶

Signaling through PDGFRb contributes to multiple tumour-associated processes, including proliferation, transvascular transport of chemotherapeutic agents, cell invasion and metastasis via autocrine, paracrine and angiogenic pathways⁴⁴ and may be a targetable downstream pathway in pancreatic cancer.⁵⁷⁵⁸ Accordingly, we present evidence for the role of PDGFRb as one possible targeted therapeutic for ARID1A-deficient tumours as it was affected by *Arid1a* re-expression, and its inhibition by imatinib dampened the invasive phenotypes observed in *KPCA^{f/f}* cell lines. Additional studies would be helpful to explore the mechanism by which ARID1A regulates PDGFRb, such as utilizing histone markers of activation and repression via ChIP-Seq or chromatin availability with ATAC-Seq.⁵⁹

Additional studies to correlate these features of ARID1A loss as a prognostic or therapeutic opportunity using human PDAC samples will also be important.

Our mouse models, *in vitro*, and transcriptomic data show that ARID1A: 1) maintains the acinar epithelia while constraining the proliferation of ductal epithelia, 2) cooperates with KRAS to accelerate the expansion of IPMN precursors and cancer formation, and 3) in the setting of *Kras* and *p53* mutations, leads to the formation of more poorly differentiated cancer associated with EMT and stem-like properties, which can be partially abrogated by *Arid1a* re-expression or PDGFRb inhibition. Our findings demonstrate the importance of ARID1A in normal pancreatic function and suppression of the development of premalignant ductal pathologies, as well as establish its role in restraining mesenchymal transition of invasive cancers.

MATERIALS AND METHODS

A detailed description of materials and methods can be found in Supplementary Materials and Methods.

Mouse lines

All animal studies were conducted in accordance with the AAALAC accredited University Committee on Animal Resources (UCAR). Experimental animals were generated using the alleles described in supplementary figure S1 and Supplementary Materials and Methods. Caerulein (Sigma) was administered by intraperitoneal injection at a dose of 250 µg/kg twice daily for two weeks. Tumour-derived cell lines were generated as previously described²¹ and genotyped as detailed in Supplementary Materials and Methods. *Arid1a* was re-expressed using the Sleeping Beauty transposon system as previously described.⁴³

Immunohistochemistry and immunofluorescence

Mouse tissue was fixed in 10% neutral buffered formalin, embedded in paraffin, and cut to 4 µm sections that were processed for staining as described in Supplementary Materials and Methods.

Quantitative PCR (qPCR) analysis

Total RNA was purified, cDNA was synthesised, and qPCR was carried out as described in Supplementary Materials and Methods.

Western blotting

SDS-PAGE was performed on whole-cell extracts and protein was detected as described in Supplementary Materials and Methods.

RNA sequencing and data analysis

RNA was isolated, sequenced, and analysed as detailed in Supplementary Materials and Methods.

Wound healing assay

Cell migration post-wounding was assessed as described in Supplementary Materials and Methods.

Clonogenic assay in soft agar

Anchorage-independent growth was evaluated as described in Supplementary Materials and Methods.

Cancer cell spheroid invasion assay⁶⁰

Spheroids were generated, and invasion was measured as described in Supplementary Materials and Methods.

Statistical analysis

Results were presented as mean $\pm$ SD and p-values were calculated using the two-tailed Student's *t* test using GraphPad Prism or Excel software. Survival was determined using the Kaplan-Meier method and comparisons between genotypes were determined using the log-rank test. Animals that were euthanised for signs of illness or were found dead were included as events with the overall survival curve. Animals that were found to have histologic evidence of cancer were included as events while those that died for reasons other than cancer were censored with the cancer-specific survival curve.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

ARID1A	AT-rich interaction domain-containing protein 1A
SWI/SNF	SWItch/Sucrose Non-Fermentable
PDAC	Pancreatic ductal adenocarcinoma
ADM	acinar-to-ductal metaplasia
IPMN	intraductal papillary mucinous neoplasm
EMT	epithelial-mesenchymal transition

PanIN	pancreatic intraepithelial neoplasia
PDGFRb	platelet-derived growth factor receptor beta
PI3K	phosphoinositide 3-kinase
ATR	ataxia telangiectasia and Rad3 related
PARP	poly(ADP-ribose) polymerase
HDAC6	histone deacetylase 6
ARID1B	AT-rich interaction domain-containing protein 1B
Ptf1a	pancreas-specific transcription factor 1a
IHC	immunohistochemistry
dpi	days post-injection
PDX1	pancreas/duodenum homeobox protein 1
IF	immunofluorescence
MIST1	muscle intestine and stomach expression 1
ERα	estrogen receptor α
MCN	mucinous cystic neoplasms
GSEA	Gene Set Enrichment Analysis
PRC2	polycomb repressive complex 2
qPCR	quantitative polymerase chain reaction
RNA-Seq	RNA sequencing
ATAC-Seq	Assay for Transposase-Accessible Chromatin using sequencing

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Significance of this study

What is already known about this subject?

- Mutations in the SWI/SNF complex are found in approximately a third of PDAC, implicating deregulation of epigenetic processes in the development and progression of this disease.
- *ARID1A* is the most frequently mutated member of the SWI/SNF complex.
- In other cancers, ARID1A loss establishes synthetic vulnerabilities in critical cellular processes including DNA damage repair, proliferation/survival signaling, and other epigenetic regulatory pathways.

What are the new findings?

- *Arid1a* deletion in the pancreas results in progressive acinar-to-ductal metaplasia with evolving pancreatitis, loss of acinar mass with pancreatic atrophy and diminished ability to regenerate acinar tissue mass in response to injury, and ductal expansion into macroscopic cystic structures.
- Mutant *Kras* cooperates with concurrent homozygous deletion of *Arid1a* leading to intraductal papillary mucinous neoplasm (IPMN), a clinically recognised PDAC precursor, instead of the predominant pancreatic intraepithelial neoplasia (PanIN) precursors seen in mutant *Kras* alone; however, these IPMNs rarely progress to fully invasive PDAC.
- *Arid1a* loss in the context of mutant *Kras* and hemizygous *p53* leads to diminished cancer-specific survival, with the resulting tumour being poorly differentiated. Cancer cell lines derived from these *Kras*; *p53*; *Arid1a* mutant models are more mesenchymal, migratory, invasive and capable of anchorage-independent growth as compared with *Arid1a*-proficient PDAC cells. Gene expression analysis of these cell lines identified enriched gene sets that suggest activation of pathways including EMT and gain of stem cell identity, including *Pdgfrb*.

How might it impact on clinical practice in the foreseeable future?

- This study sheds light on the role of ARID1A in establishing IPMN precursors in the context of two common genetic alterations in PDAC, *Kras* and *p53*.
- Gene expression analysis identifies a relationship between ARID1A loss with EMT and stem cell pathways, offering actionable targets for the development of mutation-directed therapy, such as imatinib.

Figure 1.

ARID1A maintains pancreatic homeostasis. Gross images at 52 weeks of age of (A) *C*, (B) *A^{ff}*, (C) *CA^{f/+}*, and (D) *CA^{f/f}* pancreases. Hematoxylin and eosin (H&E) staining of pancreases at 12 weeks of (E) *C*, (F) *A^{ff}*, (G) *CA^{f/+}*, (H) *CA^{f/f}* and 52 weeks of (I) *C*, (J) *A^{ff}*, (K) *CA^{f/+}*, (L) *CA^{f/f}* showing progressive acinar attrition and ductal expansion in *CA^{f/f}* compared to all controls. Immunohistochemistry (IHC) for ARID1A at 12 weeks of (M) *A^{ff}* and (N) *CA^{f/f}*. Normal acinar cells (“A”), ducts (“D”), islets (“I”), acinar-to-ductal metaplasia (“ADM”), small cysts (“SC”), and large macroscopic cysts (white arrow or asterisk). Simple columnar epithelium of a large cyst denoted with black arrow. Bar=50 μ m.

Figure 2.

ARID1A is necessary for pancreatic acinar epithelial repair following injury. (A-F) Caerulein given by intraperitoneal injection (250 μ g/kg twice daily) for two weeks to 8-week-old *A^{f/f}* and *CA^{f/f}* mice. Gross images with corresponding H&E and ARID1A IHC of pancreases at (A1-3,B1-3) 0, (C1-3,D1-3) 3, and (E1-3,F1-3) 9 days post-injection (dpi). Both (A2) *A^{f/f}* and (B2) *CA^{f/f}* show loss of acini with development of ADM (enlarged in insets) at 0 dpi. At 3 and 9 dpi, (C2,E2) *A^{f/f}* reconstitutes acinar epithelia and (D2,F2) *CA^{f/f}* shows continued acinar depletion (enlarged in insets). ARID1A IHC shows retention of ARID1A in the epithelia of *A^{f/f}* mice (A3,C3,E3) with scattered retention of ARID1A in the

epithelia of CA^{ff} mice (B3,D3,F3). (G) Quantification of percent of retained acini in A^{ff} and CA^{ff} pancreases at the indicated time points shows earlier recovery of A^{ff} pancreases compared with CA^{ff} pancreases; n=3 per genotype per time point per treatment, mean $\pm$ SD, *p<0.05, NS=not significant. Bar=50 μ m.

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Figure 3.

ARID1A is necessary for acinar cell differentiation and proliferation after injury. (A-D) PDX1 IHC of (A,C) *A^{fl/fl}* and (B,D) *CA^{fl/fl}* pancreases with (A,B) vehicle control or (C,D) caerulein treatments. Islets, re-emerging acini (denoted by black arrow), and progenitor-like ducts (denoted by outlined arrow) stain strongly for PDX1. Immunofluorescence (IF) of MIST1 and Ki-67 of caerulein-treated *A^{fl/fl}* and *CA^{fl/fl}* pancreases at (E,F) 3 and (G,H) 9 dpi separated by (E1-H1) MIST1 (green, acinar cell marker), (E2-H2) Ki-67 (red, proliferation marker), (E3-H3) Hoechst (blue, nuclear stain), and (E4-H4) overlay of all channels (white arrows denote proliferating acinar cells, ADM=acinar-to-ductal metaplasia). (I) Quantification of MIST1⁺ and Ki-67⁺ colocalised nuclei per high-power field (HPF) shows diminished MIST1 and Ki-67 co-localisation in *CA^{fl/fl}* pancreases compared to *A^{fl/fl}*

pancreases; n=3 animals per genotype per time point with 5 HPF per animal, mean $\pm$ SD, *p<0.05. Bar=50 μ m.

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Figure 4.

ARID1A restrains ductal proliferation. IF for CK19 (red, ductal marker), Ki-67 (green), and Hoechst (blue) of (A) 12-, (C), 20-, and (E) 52-week-old *Arid1a*^{f/f} and age-matched *CA*^{f/f} pancreases (B,D,F). Insets with representative duct and Ki-67⁺ cells. (G) Quantification of percent of Ki-67⁺ CK19⁺/CK19⁺ cells shows increased proliferation in *CA*^{f/f} pancreases; n=3 per genotype per time point with 3-4 HPF per animal, mean $\pm$ SD, *p<0.05, **p<0.01. Bar=50 μ m.

Figure 5. *Kras*^{G12D} and *Arid1a* loss cooperate to form IPMN. Cohorts of *Ptf1a-Cre; Kras*^{G12D} (KC), *Ptf1a-Cre; Kras*^{G12D}; *Arid1a*^{f/+} (KCA^{f/+}) and *Ptf1a-Cre; Kras*^{G12D}; *Arid1a*^{f/f} (KCA^{f/f}) were followed longitudinally. Gross images of (A) KC, (B) KCA^{f/+}, and (C) KCA^{f/f} showing large cysts in KCA^{f/f}. H&E at low magnification of (D) KC, (E) KCA^{f/+}, and (F) KCA^{f/f} mice showing a shift from PanIN to IPMN with loss of *Arid1a*. Bar=200 μ m. High magnification of (G) KC, (H) KCA^{f/+}, and (I) KCA^{f/f} mice demonstrates PanIN (G,H) and area of high grade dysplasia within IPMN (I). ARID1A IHC shows retained expression in PanIN in (J) KC, variable retention in (K) KCA^{f/+} lesions and loss of expression in (L) KCA^{f/f} IPMN. Bar=50 μ m. (M) Quantification of percent incidence of gross cysts, low-grade (LG) IPMN, high-grade (HG) IPMN, LG PanIN, and HG PanIN in mice sacrificed between the ages of 12 and 20 weeks; n=8 mice for KC, 7 mice for KCA^{f/+}, 9 mice for

KCA^{ff} , * $p < 0.05$. (N) Kaplan-Meier analysis shows decreased cancer-specific survival (CSS) in KCA^{ff} compared to KC ($p < 0.0166$, median CSS of KC (black, $n=18$) and CA^{ff} (green, $n=12$) are undefined, whereas $KCA^{f/+}$ is 75.29 weeks (blue, $n=26$), and KCA^{ff} is 62.43 weeks (red, $n=27$)).

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Figure 6.

Arid1a mutation in *Kras*^{G12D}; *p53* PDAC model. (A-C) Gross images at necropsy of (A) *Ptf1a-Cre*; *Kras*^{G12D}; *p53*^{f/+} (*KPC*), (B) *Ptf1a-Cre*; *Kras*^{G12D}; *p53*^{f/+}; *Arid1a*^{f/+} (*KPCA*^{f/+}), and (C) *Ptf1a-Cre*; *Kras*^{G12D}; *p53*^{f/+}; *Arid1a*^{f/f} (*KPCA*^{f/f}). (D-F) Low magnification H&E of (D) *KPC* PDAC with associated PanIN, (E) *KPCA*^{f/+} PDAC with mix of precursors, and (F) *KPCA*^{f/f} PDAC with associated IPMN. Bar=200 μ m. (G-I) High magnification H&E reveals (I and inset) high grade *KPCA*^{f/f} PDAC compared to (G and inset) *KPC* and (H and inset) *KPCA*^{f/+}. Bar=50 μ m. (J-L) ARID1A IHC demonstrates intact ARID1A staining in (J) *KPC* PDAC and (K) *KPCA*^{f/+} PDAC and loss of ARID1A staining in (L) *KPCA*^{f/f} PDAC. Bar=20 μ m. (M) Quantification of percent incidence of cancer, LG IPMN, HG IPMN, LG PanIN, and HG PanIN in mice sacrificed at signs of distress; n=36 mice for *KPC*, 30 mice for

KPCA^{f/+}, and 26 mice for *KPCA^{f/f}*, *p<0.05. (N) Kaplan-Meier plot of overall survival (OS) for *KPC* (black, n=36, 27.22 weeks), *KPCA^{f/+}* (blue, n=30, 25.71 weeks), and *KPCA^{f/f}* (red, n=28, 26.57 weeks) shows no significant differences in survival.

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Figure 7.

Arid1a loss increases anchorage-independent growth and migration. (A) *KPC* tumour cell lines (n=5) demonstrating epithelial morphology compared to (B) *KPCA^{f/f}* tumour cell lines (n=5) showing mesenchymal morphology. (C) Western blot of protein lysates from *KPC* and *KPCA^{f/f}* cell lines shows absence of ARID1A as expected and absence of P53 induction following cisplatin treatment in all but one *KPCA^{f/f}* cell lines. GAPDH served as a loading control. Positive control is an *Arid1a/p53* intact PDAC line (Pos ctrl). (D-F) Clonogenic assay and quantification showing (E,F) *KPCA^{f/f}* cell lines (n=2) form greater numbers of colonies in soft agar compared to (D,F) *KPC* (n=2), *p=8.5×10⁻¹³. Colonies 125 μm were counted. (G-I) Wound healing assay and quantification demonstrate that (H,I) *KPCA^{f/f}* cell lines (n=2) have increased ability to fill the wound area compared to (G,I) *KPC* (n=2) at 24 and 48 hours, **p=6.45×10⁻⁸, ***p=4.81×10⁻¹⁵. Analysis by TScratch. Bar=1000 μm for (D,E,G,H).

Figure 8.

EMT and stem cell transcriptional programs are activated in *Arid1a*-mutant PDAC. (A) Gene set enrichment analysis of differentially expressed genes from RNA-Seq of *KPC* vs *KPCA^{f/f}* using the “Hallmark” gene sets demonstrates EMT signature is the most enriched signature in *KPCA^{f/f}* lines. NES=normalised enrichment score; FDR=false discovery rate. (B,C) Gene set enrichment analysis using the “Curated chemical and genetic perturbations” gene sets demonstrates that stem cell and SUZ12 targets are highly enriched in *KPCA^{f/f}* lines. (D) Expression data of the 50 genes with the highest log2FC between *KPCA^{f/f}* and *KPC* cell lines from the gene sets in (A-C) with *Pdgrfb* highlighted in box. (E) qPCR validation of indicated genes in *KPCA^{f/f}* (n=8) compared to *KPC* (n=6) cell lines, *p<0.05.

(F) Western blot for ARID1A, E-Cadherin, and B-Tubulin in *KPCA^{ff}* and *KPC* cell lines (n=6 each) shows loss of expression of E-Cadherin in 3 of 6 *KPCA^{ff}* cell lines.

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Figure 9.

Re-expression of *Arid1a* and inhibition of PDGFRb partially abrogate the phenotypes of *KPCA^{f/f}* cell lines. (A) qPCR data showing re-expression (*KPCA^{f/f}+A*) and controls (*KPCA^{f/f}*) in three *KPCA^{f/f}* cell lines demonstrate significantly increased expression of *Arid1a* mRNA in all three lines, and significantly decreased expression of *Pdgfrb*, *Lox*, and *Lef1* in two of three lines each. (B) Quantification of wound healing assay in three *KPCA^{f/f}* cell lines with (*KPCA^{f/f}+A*) and without (*KPCA^{f/f}*) *Arid1a* re-expression demonstrates that *KPCA^{f/f}+A* have decreased ability to fill the wound area compared to controls in two of three cell lines tested. (C) Quantification of wound healing assay in three *KPC* and *KPCA^{f/f}*

cell lines treated with imatinib (1 μ M) and DMSO control demonstrates that imatinib has no effect on most *KPC* cell lines tested while imatinib-treated *KPCA^{f/f}* cell lines have decreased ability to fill the wound area compared to controls in two of three lines tested. (B,C) All measured at 24 hours, *p<0.05, analysis by TScratch. (D) Quantification of cancer cell spheroid invasion assay in three *KPCA^{f/f}* cell lines treated with imatinib (1 μ M) and DMSO control reveals decreased total area invaded by imatinib-treated *KPCA^{f/f}* cells leaving the spheroid in all three cell lines tested. All measured at 48 hours, *p<0.05, analysis by ImageJ.

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