Single-PanIN-seq unveils that ARID1A deficiency promotes pancreatic tumorigenesis by attenuating KRAS-induced senescence

Shou Liu†, Wenjian Cao‡, Yichi Niu‡, Jiayi Luo†, Yanhua Zhao, Zhiying Hu, Chenghang Zong

1Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, United States; 2Genetics and Genomics Graduate Program, Baylor College of Medicine, Houston, United States; 3Cancer and Cell, Biology Graduate Program, Baylor College of Medicine, Houston, United States; 4Dan L Duncan Comprehensive Cancer Center, Baylor College of Medicine, Houston, United States; 5McNair Medical Institute, Baylor College of Medicine, Houston, United States

ABSTRACT

ARID1A is one of the most frequently mutated epigenetic regulators in a wide spectrum of cancers. Recent studies have shown that ARID1A deficiency induces global changes in the epigenetic landscape of enhancers and promoters. These broad and complex effects make it challenging to identify the driving mechanisms of ARID1A deficiency in promoting cancer progression. Here, we identified the anti-senescence effect of Arid1a deficiency in the progression of pancreatic intraepithelial neoplasia (PanIN) by profiling the transcriptome of individual PanINs in a mouse model. In a human cell line model, we found that ARID1A deficiency upregulates the expression of aldehyde dehydrogenase 1 family member A1 (ALDH1A1), which plays an essential role in attenuating the senescence induced by oncogenic KRAS through scavenging reactive oxygen species. As a subunit of the SWI/SNF chromatin remodeling complex, our ATAC sequencing data showed that ARID1A deficiency increases the accessibility of the enhancer region of ALDH1A1. This study provides the first evidence that ARID1A deficiency promotes pancreatic tumorigenesis by attenuating KRAS-induced senescence through the upregulation of ALDH1A1 expression.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) was the third leading cause of cancer-related death in the United States in 2018 and is projected to become the second leading cause of cancer-related death by 2030 (Rahib et al., 2014). Besides the clear driver mutations of PDAC, which include CDKN2A, TP53, SMAD4, and KRAS, there are a large number of genes with low-frequency mutations (Waddell et al., 2015; Bailey et al., 2016; Cancer Genome Atlas Research Network, 2017). The statistical significance of a large number of low-frequency mutations suggests that they likely play functional roles in the promotion of tumor development. From the evolutionary perspective of tumor development, the probability of acquiring one of the mutations in low-frequency-mutation genes is significantly higher than the acquisition of one of the mutations in the very few high-frequency-mutation genes such as TP53, especially at the very early stage when the number of pre-lesion cells is limited. Therefore, it is reasonable to believe that a portion of the low-frequency mutations could be acquired at the early stage and that these mutations could play essential roles in tumorigenesis.
Among the genes with low-frequency mutations in PDAC, the top hit is ARID1A (a subunit of the SWI/SNF chromatin remodeling complex) with an 8% mutation rate (Figure 1—figure supplement 1). In addition to PDAC, ARID1A is also frequently mutated in other cancer types, with 45.2% mutation rate in ovarian cancer, 18.7% in gastric cancer, 18.6% in bladder cancer, 13.7% in hepatocellular cancer, 11.5% in melanoma, 9.4% in colorectal cancer, 8.2% in lung cancer, and 2.5% in breast cancer (Kadoch et al., 2013). Therefore, it is crucial to study the ubiquitous mechanisms for ARID1A-deficiency-facilitated tumorigenesis in various types of cancers.

ARID1A is involved in the regulation of many biological processes of cells, including differentiation, proliferation, and apoptosis (Wu and Roberts, 2013). In pancreatic cancer, recent studies have shown that ARID1A is necessary to maintain terminal differentiation of pancreatic acinar cells, and knockout of ARID1A results in the accelerated formation of acinar-to-ductal metaplasia (ADM) and then pancreatic intraepithelial neoplasia (PanIN) lesions (Zhang, 2018; Livshits et al., 2018; Wang et al., 2019). Although ARID1A depletion can prime acinar cells for early-stage PanIN lesion formation by facilitating shifts in cell identity (Livshits et al., 2018), the underlying mechanisms for the acceleration of ARID1A-deficiency-promoted PanIN progression remain elusive. Besides PanIN lesions, ARID1A deficiency could also facilitate intraductal papillary mucinous neoplasm (IPMN) formation through multiple pathways, including MYC-mediated protein synthesis (Wang et al., 2019) and SOX9/mTOR pathway (Kimura et al., 2018).

To dissect the mechanisms whereby Arid1a knockout drives PanIN progression, we applied the single-cell RNA-seq method to profile the transcriptome of individual early-stage PanIN lesions from Arid1a knockout and wildtype mice. Our results showed that Arid1a knockout could effectively reduce KRAS-induced senescence in PanIN lesions. It is important to point out that cellular senescence has been shown to be the major rate-limiting step in KRAS-driven PanIN progression (Morton et al., 2010; Serrano et al., 1997; Li et al., 2018). Therefore, with significant attenuation of senescence, Arid1a knockout can achieve significant acceleration of PanIN progression. Mechanistically, we found that aldehyde dehydrogenases play an essential role in attenuating senescence by scavenging the reactive oxygen species (ROS) induced by oncogenic KRAS.

**Results**

**Individual PanIN lesion RNA-Seq unveils the potential player contributing to the attenuation of Kras-induced senescence in Arid1a knockout mice**

To identify the mediators that contribute to Arid1a-deficiency-promoted PanIN progression, we followed the PanIN progression in conditional Arid1a knockout mice with mutant Kras (Arid1a<sup>fl/fl</sup>;Lox-Stop-Lox-Kras<sup>G12D/+;Ptf1a<sup>CreERT/</sup></sup>, we abbreviate it as AKC) and mice without Arid1a knockout (Arid1a<sup>+/+</sup>;Lox-Stop-Lox-Kras<sup>G12D/+;Ptf1a<sup>CreERT/</sup></sup>, we abbreviate it as KC) (Figure 1—figure supplement 2A). Consistent with the findings from other groups (Zhang, 2018; Livshits et al., 2018; Wang et al., 2019), we observed that Arid1a knockout facilitated the progression of lesions from ADM to PanIN3 (Figure 1—figure supplement 2B,C). At as early as the 2-month time point, the percentages of ADM, PanIN-1, and PanIN-2 were 74, 26, and 0%, respectively, in KC mice versus 53, 47, and 0% in AKC mice. At the 6-month time point, the percentages of ADM, PanIN-1, PanIN-2, and PanIN-3 were 77, 23, 0, and 0%, respectively, in KC mice versus 16, 74, 9, and 0.5% in AKC mice (Figure 1—figure supplement 2C). We observed the decrease in the percentage of ADM and increase in the percentage of high-grade PanIN lesions, indicating the accelerated progression of lesions.

To profile the transcriptome of individual PanIN lesions, we combined laser capture microdissection (LCM) with a highly sensitive single-cell RNA-seq method (MATQ-seq) developed in our lab (Sheng et al., 2017; Figure 1A). In total, we dissected and profiled 20 lesions from two KC mice and 24 lesions from two AKC mice. We only dissected PanIN-1 and PanIN-2 lesions because their duct-like structures can be easily recognized for dissection from frozen sections (Figure 1—figure supplement 3A). We observed that the lesions dissected from KC and AKC mice were clearly separated in the multidimensional scaling (MDS) plot, and 861 differentially expressed genes (DEGs) (Supplementary file 2), including 532 genes upregulated and 329 genes downregulated in AKC, were identified, which suggests the successful transcriptome profiling of individual PanIN lesions by MATQ-seq (Figure 1B).
Figure 1. Single pancreatic intraepithelial neoplasia (PanIN) lesion RNA-seq unveils the potential player contributing to the attenuation of Kras-induced senescence in Arid1a knockout mice. (A) Experimental scheme of transcriptome profiling of single PanIN lesions. (B) Multidimensional scaling plot showed a clear separation of the transcriptome profiles of lesions from AKC mice (Arid1a\^fl/fl; LSL-Kras\^G12D/+; Ptf1a\^CreERT/+) and KC mice (Arid1a\^+/+; LSL-Kras\^G12D/+; Ptf1a\^CreERT/+). 24 lesions from 2 AKC mice and 20 lesions from 2 KC mice were used for single-lesion RNA sequencing. (C) Hallmark gene sets that are significantly enriched between lesions from AKC and KC mice. (D, E) Enrichment plots of P53_PATHWAY and SENESCENCE_UP from gene set enrichment analysis. 

The online version of this article includes the following figure supplement(s) for figure 1:

Figure supplement 1. Mutations in ARID1A in ICGC-PACA-AU and TCGA pancreatic ductal adenocarcinoma (PDAC) cohorts based on cBioPortal data portal.

Figure supplement 2. Arid1a knockout drastically accelerates pancreatic intraepithelial neoplasia (PanIN) progression.

Figure supplement 3. Profile of the transcriptomes of individual pancreatic intraepithelial neoplasia (PanIN) by PanIN-seq.

Figure supplement 4. The gene set enrichment plots of 27 relevant pathways with statistical significance in Afl/fl KC lesions.
With DEGs observed between AKC and KC lesions, we performed gene set enrichment analysis (GSEA) using Hallmark gene sets (Liberzon et al., 2015) to interrogate the pathways perturbed by Arid1a knockout. We observed that 25 gene sets were downregulated, and surprisingly, only two gene sets were upregulated in AKC lesions (Figure 1C, Figure 1—figure supplement 4, and Supplementary file 1, false discovery rate (FDR) < 0.1). It is worth noting that among the 27 gene sets 2 are specifically associated with Kras activation: KRAS_SIGNALING_UP (the gene set upregulated upon Kras activation) and KRAS_SIGNALING_DN (the gene set downregulated upon Kras activation). The gene set KRAS_SIGNALING_UP was upregulated while the gene set KRAS_SIGNALING_DN was downregulated (Figure 1C and Figure 1—figure supplement 4). This observation suggests that the activities of Kras signaling are partially impaired by Arid1a deficiency.

Furthermore, we observed that the Tp53 signaling pathway was suppressed in AKC lesions (Figure 1D). It has been well established that upregulation of the Tp53-related pathway is closely associated with apoptosis or senescence. ARID1A mutations have also been shown to be mutually exclusive with TP53 mutations in endometrial cancer (Wu et al., 2017). To determine whether Arid1a is involved in the regulation of apoptosis, senescence, or both, we further examined the activity of related pathways in Arid1a KO lesions. Interestingly, we found that the senescence-associated signaling pathway is significantly suppressed in lesions from AKC mice (Figure 1E). In contrast, the pathway activity associated with apoptosis was not significantly changed (Figure 1—figure supplement 3B). These observations led us to hypothesize that Arid1a deficiency could promote PanIN lesion progression through the attenuation of Kras-induced senescence.

Furthermore, senescent cells feature senescence-associated secretory phenotype (SASP), including high levels of inflammatory cytokines and immune modulators. With the attenuation of senescence promoted by Arid1a deficiency, we expected to observe reduced inflammatory response in the GSEA. Indeed, we observed that multiple signaling pathways associated with inflammation, including TNFα signaling, IL6–STAT3 signaling, IL2–STAT5 signaling, IFN-γ signaling, and IFN-γ signaling, were significantly suppressed in Arid1a KO lesions (Figure 1C, Figure 1—figure supplement 4, and Supplementary file 1).

**In vivo, ex vivo, and in vitro verification of the attenuation of Kras-induced senescence by Arid1a deficiency**

To verify the effect of Arid1a deficiency on Kras-induced senescence, we performed senescence-associated beta-galactosidase (SA-β-Gal) staining on lesions from KC and AKC mice. SA-β-Gal-positive lesions were observed in five out of seven (71%) KC mice. In contrast, only one out of six (17%) AKC mice showed SA-β-Gal-positive lesions. Among the mice with SA-β-Gal-positive lesions, the percentage of SA-β-Gal-positive lesions in KC mice was about twice of that in AKC mice (Figure 2A,B). These data confirmed that Arid1a knockout indeed reduced Kras-induced senescence.

To further verify the effects of Arid1a knockout on senescence, we performed an ex vivo culture experiment using acinar cells isolated from AKC and KC mice. SA-β-Gal staining was performed to examine the senescence of acinar cells. As shown in Figure 2C,D, due to flat morphology of senescent cells and the large cell-size variation, we cannot accurately quantify the number of SA-β-Gal-negative cells. Instead of using the percentage of senescence cells, here we quantified the senescence based on the intensity of SA-β-Gal staining of the positive cells. We observed that the intensity of SA-β-Gal staining in acinar cells from AKC mice was significantly less than that from KC mice. Importantly, this result suggests that the effect of Arid1a knockout on senescence is likely an intrinsic cellular response rather than a microenvironmental response such as accelerated clearance of senescent cells by immune cells (Pignolo et al., 2020; Baker et al., 2011).

Based on the results of the ex vivo experiment, we next tested whether we could observe similar effects of ARID1A deficiency on KRAS-induced senescence in vitro cell lines. We established a human pancreatic Nestin-expressing (HPNE) cell line (an intermediary cell type formed during ADM) with inducible KRAS (G12D) expression (iKRAS-HPNE cells). The induction of KRAS activity was verified by western blot of both KRAS and phosphorylated ERK (Figure 2—figure supplement 1A). Next, we knocked out ARID1A by CRISPR-Cas9 in iKRAS-HPNE cells. Two isogenic HPNE clones were used in the following experiments (Figure 2—figure supplement 1B), and the knockout efficiency of ARID1A was further confirmed by qRT-PCR (Figure 2—figure supplement 1C).
To examine the effect of ARID1A deficiency on KRAS-induced senescence, we first performed SA-β-Gal staining in ARID1A knockout (ARID1A-KO) and wildtype iKRAS-HPNE cells upon KRAS induction. We observed that ARID1A-KO cells showed a slightly lower percentage of SA-β-Gal-positive cells compared with wildtype iKRAS-HPNE cells (Figure 2—figure supplement 1D). The in vitro result of SA-β-Gal staining is less significant than what we observed both in vivo and ex vivo. One possible reason for this discrepancy is that in terms of evaluation of senescence SA-β-Gal staining may
not be as effective in immortalized cell lines as in tissue samples (Dimri et al., 1995). Colony formation assay is an alternative method to measure senescence. Here, we performed a colony formation assay to evaluate the ability of cells to escape from KRAS-induced senescence. We observed that knockout of ARID1A drastically increased the number of cells escaping from KRAS-induced senescence (Figure 2E,F).

ARID1A knockout reduces KRAS activities and inflammatory response

With the consistent in vivo, ex vivo, and in vitro observation of the anti-senescence effects of ARID1A deficiency, we next investigated the molecular mechanisms by which ARID1A knockout promotes the escape from KRAS-induced senescence. We first performed RNA-seq on ARID1A-KO (clone #2) and wildtype HPNE cells with or without KRAS induction. We observed a clear separation between ARID1A-KO and wildtype HPNE cells under both conditions in the MDS plot (Figure 3A).

Next, we performed GSEA using the Hallmark gene sets. Interestingly, we found the gene set KRAS_SIGNALING_UP was downregulated in ARID1A-KO cells compared with wildtype cells (Figure 3—figure supplement 1A,B), which is consistent with our data from PanIN lesions. To verify this observation, we examined the activity of ERK, a classical downstream effector of KRAS signaling, in ARID1A-KO and wildtype cells. As shown in Figure 3—figure supplement 2, the phosphorylation of ERK upon KRAS induction in ARID1A-KO cells was significantly reduced in comparison with the wildtype cells. The consistent observation between in vivo and in vitro indicates that ARID1A deficiency partially impairs the activities of KRAS signaling.

Next, we performed an interaction test on the gene expression data to identify the genes that have different responses to KRAS activation depending on ARID1A status (Supplementary file 4). GSEA results showed that six signaling pathways were remarkably repressed in ARID1A-KO HPNE cells, and four of them are involved in inflammatory response (Figure 3—figure supplement 3A,B), which is consistent with what we observed in PanIN lesions from AKC mice (Figure 1C).

Furthermore, we examined the expression of a classic marker of cellular senescence: CDKN1A. We observed that the activation of CDKN1A expression upon KRAS induction was also significantly reduced in the ARID1A-KO cell line (Figure 3—figure supplement 3C,D), which indicates that our HPNE cell line model successfully recapitulated the senescence phenotypes observed in the mouse model.

ARID1A knockout significantly upregulates the expression of aldehyde dehydrogenase (ALDH) family members

To identify the underlying players that promote the attenuation of cellular senescence, we analyzed the DEGs between wildtype and the ARID1A-KO cell line with or without KRAS induction (Figure 3B, Figure 3—figure supplement 4A, and Supplementary file 3). For the first clone with KRAS induction, we identified 125 upregulated genes and 165 downregulated genes between the wildtype and the ARID1A-KO line (Figure 3B). To exclude the genes whose expression changes may be associated with mutant KRAS signaling, we compared the list of DEGs under two conditions: with or without KRAS induction. As shown in Figure 3C, for the upregulated genes, 57 out of 125 genes (46%) are shared between the two conditions and the expected number of random overlap is 1.79. For the downregulated genes, 54 out of 165 genes (33%) are shared and the expected number of random overlap is 3.38. These results indicate that these genes are mainly dependent on ARID1A deficiency.

Among the DEGs between ARID1A-KO and wildtype HPNE cells, ALDH1A1 exhibits the significant change in differential gene expression for both conditions: with or without KRAS induction (Figure 3B and D, Figure 3—figure supplement 4A, and Supplementary file 3). To rule out any potential clonal bias, we also performed RNA-seq on a second clone (clone #11). We observed that ALDH1A1 was also significantly upregulated in the second clone under both conditions (Figure 3—figure supplement 4B–D and Supplementary file 3). The upregulation of ALDH1A1 in ARID1A-KO cells was further verified by both qRT-PCR (Figure 3—figure supplement 4E) and western blot (Figure 3E). Considering that ALDH1A1 has been shown to participate in the clearance of ROS (Raha et al., 2014) and ROS are vital mediators of KRAS-induced senescence (Storz, 2017), we hypothesize that ALDH1A1 is the gene that mediates the effect of ARID1A deficiency on KRAS-induced senescence.

Next, we examined our PanIN-seq data to evaluate the expression of Aldh1a1 and other members of the ALDH family. Interestingly, we observed that Aldh3a1 is significantly
Figure 3. ARID1A knockout upregulates aldehyde dehydrogenase (ALDH) expression. (A) Multidimensional scaling plot demonstrated clear separation between the transcriptome profiles of ARID1A-KO human pancreatic Nestin-expressing (HPNE) cells and wildtype cells with or without KRAS induction. RNA sequencing was performed with three biological repeats. (B) Volcano plot of differentially expressed genes between ARID1A knockout cells and wildtype cells with KRAS induction. (C) Venn diagram showing the upregulated genes (upper) and downregulated genes (bottom) that are shared.

Figure 3 continued on next page
between cells with (gray) or without (blue) KRAS induction. (D) ALDH1A1 mRNA levels quantified by sequencing data are significantly different between ARID1A-KO cells and wildtype cells with (left) or without (right) KRAS induction. CPM: count per million reads. (E) Western blot for ALDH1A1 expression in ARID1A-KO cells and wildtype cells with KRAS induction. (F) mRNA level of Aldh3a1 in KC and AKC lesions based on pancreatic intraepithelial neoplasia (PanIN)-seq data. APM: amplicon per million reads. (G) IHC staining against ALDH3A1 in KC and AKC lesions. Scale bars: 200 µm. (H) Comparison of ALDH3A1 levels between KC and AKC lesions based on the intensity of staining in (G). H-score was calculated by counting the number of lesions with different levels of staining intensity at four random fields under the microscope. Student’s t-test: ***p<0.001; ****p<0.0001.

The online version of this article includes the following figure supplement(s) for figure 3:

**Figure supplement 1.** Gene set enrichment analysis on RNA-seq data.
**Figure supplement 2.** ARID1A knockout impairs phosphorylation of ERK in human pancreatic Nestin-expressing (HPNE) cells upon KRAS induction.
**Figure supplement 3.** Differential response to oncogenic KRAS in ARID1A knockout impairs phosphorylation of ERK in human pancreatic Nestin-expressing (HPNE) cells upon KRAS induction.

**Figure supplement 4.** ALDH1A1 expression in ARID1A knockout human pancreatic Nestin-expressing (HPNE) cells.

upregulated in lesions from AKC mice (Figure 3F and Supplementary file 2), which was further confirmed by immunohistochemistry (IHC) staining (Figure 3G,H). This result suggests that in different species different types of ALDH family proteins can be used to mediate the attenuation of Kras-induced senescence in Arid1a-deficient cells.

**ARID1A KO facilitates escape from KRAS-induced senescence via ALDH1A1**

Given the important role of ALDH in ROS clearance, a high level of ALDH could also be important for the development of KRAS-driven PDAC. Here, we analyzed the expression of ALDH family members in normal pancreas and PDAC samples (Bailey et al., 2016; GTEx Consortium, 2013). In normal pancreas tissues, we mainly observed the expression of ALDH1A1 (Figure 4—figure supplement 1A), with different cell types exhibiting different expression levels of ALDH1A1 (Figure 4—figure supplement 1B). Since the tumor cells are mainly epithelial cells, we only compared PDAC data to pancreatic ductal cells to avoid the confounding factors caused by the cell type difference. As shown in Figure 4—figure supplement 1B, there are four subclusters of normal ductal cells. The average expression level of ALDH1A1 in normal pancreatic ductal cells (clusters 1–3) is less than 50. We excluded cluster 4 since the ALDH1A1-positive cells are indicative of the ductal stem cell population (Rovira et al., 2010). In contrast to the expression levels in normal ductal cells, we observed that in 63% of PDAC samples, the expression levels of ALDH1A1 are higher than 50 TPM, and in 10% of samples, the expression levels are higher than 200 TPM (Figure 4 and Figure 4—figure supplement 1C). Furthermore, we examined the mutation levels in ALDH1A1. We observed that only 0.2% of the patients (1 out of 576 patient samples from two cohorts [Bailey et al., 2016; Cancer Genome Atlas Research Network, 2017]) acquired mutations in ALDH1A1 (Figure 4B). This observation further supports our hypothesis that ALDH1A1 plays an important role in KRAS-driven PDAC development.

Next, to validate the essential role of ALDH1A1 in promoting the escape of cells from KRAS-induced senescence, we performed a colony formation assay in HPNE cells with and without N,N-diethylaminobenzaldehyde (DEAB, a pan-inhibitor of ALDH) treatment. We observed that inhibition of ALDH1A1 activity significantly decreased the number of colonies formed in ARID1A knockout cells; in contrast, no significant changes were observed in the wildtype cells (Figure 4C,D). To rule out the unknown effects of DEAB on HPNE cells, we also performed a colony formation assay on ARID1A-KO HPNE cells with and without ALDH1A1 knockdown. The knockdown efficiency was verified by qRT-PCR (Figure 4—figure supplement 2). We also observed that the colony number in ARID1A-KO cells with ALDH1A1 knockdown was significantly less than that without ALDH1A1 knockdown (Figure 4E,F), which is consistent with the results of the ALDH inhibitor experiment.

Furthermore, we examined the levels of ROS production in ARID1A-KO cells and wildtype cells. We observed that the fraction of ROS-positive cells in ARID1A-KO iKRAS-HPNE cells was significantly less than in wildtype cells, regardless of KRAS induction (Figure 4G). To verify the role of ALDH1A1 in reducing ROS, we measured the ROS level in ARID1A-KO cells with ALDH1A1 knockdown. We observed that the ROS level in ALDH1A1 knockdown cells was significantly higher than that observed in the cells with scramble shRNA (Figure 4H), indicating that ALDH1A1 is responsible for the clearance of ROS in HPNE cells with ARID1A knockout. From the results above, we conclude that ARID1A...
knockout can effectively reduce cellular ROS levels by upregulating the expression of ALDH1A1, which then leads to significant attenuation of KRAS-induced senescence and acceleration of PanIN progression (Figure 4I).

Figure 4. ARID1A knockout facilitates escape from KRAS-induced senescence by upregulating ALDH1A1 expression. (A) Heatmap of the expression levels of aldehyde dehydrogenase (ALDH) family members in pancreatic ductal adenocarcinoma (PDAC) patients (Bailey et al., 2016). (B) Mutation rates of ALDH1A1, ALDH1A3, and ALDH3A1 in PDAC patients. The mutation data from these two studies (Bailey et al., 2016) and TCGA (Cancer Genome Atlas Research Network, 2017) were used for analysis. (C) Colony formation assay of ARID1A knockout cells and wildtype cells with KRAS induction, treated with and without ALDH inhibitor DEAB. (D) Quantification of colony number in panel (C). The colony formation assay was performed twice. (E) Colony formation assay of ARID1A knockout cells expressing shRNA targeting ALDH1A1 and scramble shRNA control. (F) Quantification of colony number in panel (E). The colony formation assay was performed twice. (G) Measurement of the reactive oxygen species (ROS) level using an H2DCFDA-based ROS detection assay kit. Percentage of positive cells measured by flow cytometry. (H) Measurement of the ROS levels in the ARID1A-KO cells with ALDH1A1 knockdown or scramble shRNA. (I) Working model for ARID1A-deficiency-promoted pancreatic intraepithelial neoplasia (PanIN) lesion progression via inhibition of ROS production. Student’s t-test: *p<0.05; **p<0.01; ***p<0.001.

The online version of this article includes the following figure supplement(s) for figure 4:

Figure supplement 1. The expression of aldehyde dehydrogenase (ALDH) family members in normal pancreas and pancreatic ductal adenocarcinoma (PDAC).

Figure supplement 2. Knockdown efficiency of ALDH1A1 in human pancreatic Nestin-expressing (HPNE) cells with ARID1A knockout (clone #2 and #11) was confirmed by qRT-PCR.
ARID1A KO alters gene expression mainly by modulating the chromatin accessibility of distal regulatory elements

As one of the DNA binding subunits of the SWI/SNF complex, ARID1A can regulate gene expression by modulating the chromatin accessibility for transcription factor (TF) binding, recruitment of coactivators/corepressors, and facilitation of chromatin looping required to approximate promoters with distal enhancers (Wu and Roberts, 2013). To investigate how ARID1A deficiency enhances the expression of ALDH1A1, we performed ATAC-seq on ARID1A-KO and wildtype HPNE cells. The distribution of the fragment sizes and the distance from transcription start sites for both wildtype and ARID1A-KO are shown in Figure 5—figure supplement 1A–F (Supplementary file 5). As shown in Figure 5A, the Spearman correlation coefficients between knockout and wildtype cells are significantly lower than those between replicate samples in both groups (Figure 5B and Figure 5—figure supplement 1G), suggesting that the changes in DNA accessibility were robustly captured.

Next, we examined the accessibility of the promoter and enhancer regions of the genes that are differentially expressed between ARID1A-KO cells and wildtype cells. We separated the DEGs into two groups based on the fold change. The genes with positive fold-change values are the genes upregulated in ARID1A-KO cells (upregulated genes group in Figure 5B), and the genes with negative fold-change values are the genes downregulated in ARID1A-KO cells (downregulated genes group in Figure 5B). We plotted a scatter plot of read counts in peaks between wildtype and ARID1A-KO for promoters (Figure 5C) and enhancers (Figure 5D) and observed that the number of peaks affected by ARID1A deficiency in the distal regulatory regions is significantly larger than the number of peaks in promoter regions.

The heatmap of reads for the differential peaks is shown in Figure 5E, and the average read density profiles are shown in Figure 5—figure supplement 2A,B. For the differential peaks of promoters, the heatmaps of reads and the average read density profiles are shown in Figure 5—figure supplement 2C,D. We also performed analysis for the distribution of the differential peaks (Figure 5—figure supplement 3A,B) and a functional enrichment analysis (Figure 5—figure supplement 3C,D) using the GREAT algorithm (McLean et al., 2010). We observed consistent enrichment in enhancer regions. Significant interactions between the SWI/SNF complex and distal regulatory regions have also been observed in colon cancer (Mathur et al., 2017).

Next, we examined the association between DEGs and the peaks with differential accessibility. We observed that the number of DEGs is associated with peak changes with statistical significance for both promoters and enhancers (Figure 5—figure supplement 4A–D). We also noticed that the number of DEGs associated with peak changes in enhancer elements is significantly larger than the number of genes associated with peak changes in the promoter regions. This result further supports that ARID1A knockout alters gene expression mainly by modulating the chromatin accessibility of the enhancer elements.

Considering the observation that ARID1A deficiency impairs the activities of KRAS signaling pathways based on the GSEA of transcriptome data (KRAS_SIGNALING_UP in Figure 3—figure supplement 1A), next we examined the chromatin accessibility of the genes involved in KRAS signaling. We observed that in comparison to the wildtype cells, chromatin accessibility decreased in ARID1A-KO cells (Figure 5—figure supplement 5). This observation suggests that ARID1A deficiency impairs the activities of the KRAS signaling pathways by partially impairing the chromatin accessibility of the genes downstream of the KRAS pathways.

We next performed motif enrichment analysis for the differential ATAC peaks. We separated the ATAC peaks with significant changes between ARID1A-KO and wildtype cells into four groups: distal peaks with increased accessibility in ARID1A-KO cells, distal peaks with decreased accessibility in ARID1A-KO cells, promoter peaks with increased accessibility in ARID1A-KO cells, and promoter peaks with decreased accessibility in ARID1A-KO cells. We then performed motif enrichment analysis by using the AME algorithm (McLeay and Bailey, 2010) on each group of ATAC peaks. Interestingly, we found that the binding motifs of SRY, FOX family, CDX1, SOX5, etc., were enriched in the ATAC peaks with significantly increased accessibility in ARID1A-KO cells (Figure 5F, Figure 5—figure supplement 6A,B and Supplementary file 7). The binding motifs of the FOS-JUN family, NF2L1, etc., were significantly enriched in the ATAC peaks with significantly decreased accessibility (Figure 5G, Figure 5—figure supplement 6B, and Supplementary file 7), which is consistent with the results of a recent study showing ARID1A as a co-factor of AP-1 (Sen et al., 2019).
Figure 5. ARID1A knockout activates transcription of the ALDH1A1 gene by increasing the accessibility of its enhancer region. (A) Spearman correlation coefficients of the read counts in peaks between ARID1A-KO human pancreatic Nestin-expressing (HPNE) cells and wildtype cells. ATAC sequencing was performed with three biological repeats. (B) The average fold change of chromatin accessibility in the promoters and enhancers of differentially expressed genes identified in RNA-seq. Mann–Whitney–Wilcoxon test: ****p<0.0001. (C, D) The scatter plot of the read counts in each peak between ARID1A-KO cells and wildtype cells for promoter (C) and distal regions (D). The peaks with significantly increased read density in ARID1A-KO cells

Figure 5 continued on next page
ARID1A KO activates transcription of the ALDH1A1 gene by increasing the accessibility of its enhancer region

To understand how the gene expression of ALDH1A1 is altered by ARID1A knockout, we examined the accessibility of the promoter and distal regulatory elements for the ALDH1A1 gene in both ARID1A-KO and wildtype cells. Interestingly, we found that there was a significant increase in accessibility in 9 out of 11 peaks at the distal regions when we compared ARID1A-KO cells with wildtype cells (Supplementary file 6). To further verify these functional regions, we compared the landscape of two well-known enhancer markers (H3K27ac and H3K4me1) in seven highly expressed ALDH1A1 cell lines (ALDH1A1 high cell lines) (Mei et al., 2017) with our ATAC-seq peaks (Supplementary file 8). We observed that five enhancer loci clearly overlapped with the active enhancer markers (Figure 6A and Figure 6—figure supplement 1A,B).

We also plotted out the landscape of H3K27ac and H3K4me1 in five lowly expressed ALDH1A1 cell lines (ALDH1A1 low cell lines) (Mei et al., 2017), and we did not observe any significant overlap between our ATAC-seq peaks and H3K27ac/H3K4me1-enriched regions (Figure 6A). For the five enhancer loci, we observed a consistent increase in accessibility in ARID1A knockout cells in comparison with wildtype cells (Figure 6B). Overall, these results confirm that ARID1A deficiency upregulates ALDH1A1 expression by increasing the accessibility of the associated enhancer elements.

To identify proteins that could potentially bind to these five enhancer loci, we examined the TF ChIP-seq datasets from seven ALDH1A1 high cell lines and five ALDH1A1 low cell lines (Mei et al., 2017). We counted the binding events of each TF in the five candidate enhancer peaks for both ALDH1A1 high cell lines and ALDH1A1 low cell lines (Supplementary file 9). We list the TFs that could bind to the enhancer regions in Figure 6C. The TFs whose binding events were preferentially detected in the datasets from ALDH1A1 high cell lines (Fisher’s test, p<0.05) are indicated in red and include EP300 and NR3C1. For the TFs that do not have enough datasets for a statistical test, we indicated the TFs whose binding events are observed in more than 50% of ALDH1A1 high cell line datasets in blue, and the TFs whose binding events are observed in more than 25% but less than 50% of ALDH1A1 high cell line datasets in black (Figure 6C, left panel). The TFs that are not expressed in HPNE cell lines were removed.

To verify the regulation of ALDH1A1 expression by the TFs identified above, we knocked down EP300 and NR3C1, respectively, in ARID1A-KO HPNE cell line (Figure 6—figure supplement 2A,B). In Figure 6C (middle and right panels), we observed that both knockdowns could significantly impair the transcription of the ALDH1A1 gene. Collectively, these data also indicate that ARID1A deficiency promotes active transcription of ALDH1A1 by altering genome accessibility and therefore allowing the binding of EP300 and/or NR3C1 to the corresponding enhancer loci.
Figure 6. ARID1A knockout activates transcription of the ALDH1A1 gene by increasing the accessibility of its enhancer region. (A) The ATAC-seq tracks and H3K4me1/H3K27ac ChIP-seq tracks of the distal regions of the ALDH1A1 gene. The ChIP-seq tracks from different cell lines are labeled in different colors and overlaid. The figure with separated tracks is shown in Figure 6—figure supplement 1. The ALDH1A1high cell lines include A549, LOUCY, A673, 22RV1, VCAP, K562, and HepG2. The ALDH1A1low cell lines include HCT116, MCF7, Panc1, PC9, and DOHH2. The ChIP-seq data were obtained from the ENCODE database (ENCODE Project Consortium, 2012). (B) Read counts in the five enhancer peaks of the ALDH1A1 gene in ARID1A-KO human pancreatic Nestin-expressing (HPNE) cells and wildtype cells. p-value: *p<0.05; ***p<0.001; ****p<0.0001. (C) Transcription factors (TFs) that regulate ALDH1A1 expression by binding to its enhancer loci. Left panel: the list of TFs that can potentially bind to the enhancer regions of ALDH1A1 based on the ChIP-seq data from seven ALDH1A1high cell lines and five ALDH1A1low cell lines. The TFs whose binding events are preferentially detected Figure 6 continued on next page
Discussion

As a bona fide oncogenic driver of pancreatic cancer, the mechanism by which mutant KRAS counteracts the oncogenic stress that it induces is critically important for the progression of precursor lesions (Storz, 2017). It has been shown that KRAS can upregulate the expression of multiple oxidoreductases via NRF2 (nuclear factor, erythroid derived 2, like 2) to counteract oncogenic stress (DeNicola et al., 2011). Meanwhile, KRAS can also enhance NADPH production by reprogramming the metabolism of glutamine to assist oxidoreductases in scavenging ROS (Son et al., 2013). However, the observation that a high percentage of senescent PanIN lesions occur in the pancreas of KC mice suggests that the mechanisms described above likely have limited effects in reducing Kras-induced ROS in neoplastic lesions. In the early stage of PDAC development, it is plausible that new mutations can be acquired to help cells to reduce ROS and escape the oncogenic Kras-induced senescence.

In this study, we successfully adapted a high-sensitivity total-RNA-based single-cell RNA-seq method, MATQ-seq, to profile the transcriptome of single lesions. We showed that transcriptome profiling of individual lesions is not only technically feasible but also it can provide important insights about the potential mechanisms of tumor progression. The transcriptome profiling of early lesions directly led us to unveil the effects of ARID1A knockout in attenuating Kras-induced senescence and identify the important roles of ALDH1A1 in mitigating the ROS stress induced by oncogenic KRAS.

It is worth noting that ARID1A has also been linked to the regulation of ROS through other pathways. Sun et al. found that ARID1A overexpression causes an increase in ROS by activating transcription of cytochrome P450 enzymes (CYP450) at the initiation stage of liver cancer (Sun et al., 2017). Interestingly, Ogiwara et al. found that ARID1A deficiency results in elevated ROS by inhibiting the transcription of SLC7A11 (a transporter gene required for the import of cystine and the production of glutathione) in ovarian cancer (Ogiwara et al., 2019). Therefore, the effects of ARID1A deficiency on ROS are likely tissue-specific.

Besides reducing ROS levels through the upregulation of ALDH family proteins, we also observed that ARID1A deficiency directly suppresses the activities of KRAS signaling. Our in vitro and in vivo data showed that the activation of Hallmark gene sets KRAS_SIGNALING_UP and KRAS_SIGNALING_DN is remarkably impaired upon ARID1A knockout. Consistently, the activation of several inflammation-related signaling pathways upon Kras induction is inhibited in PanIN lesions and HPNE cells with ARID1A deficiency compared with wildtype counterparts, including TNFα signaling, IL6–STAT3 signaling, IL2–STAT5 signaling, IFN-α signaling, and IFN-γ signaling. Both reduction of KRAS activities and inflammatory cytokine response are also consistent with our observation of the increased attenuation of Kras-induced senescence upon ARID1A knockout.

Overall, experimental evidence shows that mutant KRAS signaling needs to be tuned to efficiently drive tumorigenesis: too much signaling could lead to cell growth arrest, while too little signaling could limit proliferation (Li et al., 2018). To find the ‘sweet spot’ of KRAS activation, KRAS could harness its own activities by changing the mutation pattern (including mutation position and type of substitution) and seeking cooperation with other genes (e.g., mutagenesis of tumor suppressors) through the course of passive selection during tumor development (Li et al., 2018). Overall, our study demonstrates the important roles of epigenetic mutations in harnessing KRAS activities and promoting tumorigenesis. Future studies of such effects in other frequently mutated epigenetic genes are greatly desired.
### Materials and methods

#### Key resources table

| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|-----------------------------------|-------------|---------------------|-------------|------------------------|
| Genetic reagent (Mus musculus)    | Ptf1a<sup>CreERT</sup> | Jackson Lab | No: 019378 |                        |
| Genetic reagent (M. musculus)     | Lox-Stop-Lox-Kras<sup>G12D</sup> | Jackson Lab | No: 008179 | Dr. Zhong Wang lab (University of Michigan) |
| Genetic reagent (M. musculus)     | Arid1a<sup>lox</sup> | PMID:18448678 | |                        |
| Cell line (Homo sapiens)          | hTERT-HPNE | Dr. Jennifer Baily lab (UT Health) | RRID:CVCL_C466 |                        |
| Recombinant DNA reagent           | pL-Inducer20-<sup>G12D</sup> | Dr. Haoqiang Ying lab (MD Anderson Cancer Center) | | Inducible expression of KRAS<sup>G12D</sup> |
| Recombinant DNA reagent           | pL-CRISPR.EFS.tRFP | Addgene | RRID:Addgene_57819 | Pol III-based sgRNA expression backbone |
| Recombinant DNA reagent           | pGIPZ | Open Biosystems | | Pol III-based shRNA expression backbone |
| Sequence-based reagent            | sgARID1A | This paper | sgRNA | CAGCGGTACCCGATGACCAT |
| Sequence-based reagent            | shALDH1A1 | This paper | shRNA | GGAGTGGTTACAAAGACATT |
| Sequence-based reagent            | shEP300-1 | This paper | shRNA | CGGCAAAACAGTTGTGCACA |
| Sequence-based reagent            | shEP300-2 | This paper | shRNA | AGCTACTGAGGTAGTTA |
| Sequence-based reagent            | shNR3C1-1 | This paper | shRNA | CCAACGCTGGCATTGTGA |
| Sequence-based reagent            | shNR3C1-2 | This paper | shRNA | AGCTGTAAGTTTCTTCA |
| Commercial assay or kit           | ROS detection assay kit | BioVision | Cat# K936-250 |                        |
| Commercial assay or kit           | Senescence β-Galactosidase Staining Kit | Cell Signaling Technology | Cat# 9860 |                        |
| Chemical compound, drug           | ALDEFLUOR diethylaminobenzaldehyde (DEAB) reagent, 1.5 mM in 95% ethanol | Stemcell Technologies Inc | Cat# 01705 |                        |
| Antibody                          | Rabbit polyclonal anti-ALDH1A1 | Abcam | Cat# ab23375, RRID:AB_2224009 | WB(1:400) |
| Antibody                          | Rabbit polyclonal anti-Aldh3a1 | Abcam | Cat# ab76976, RRID:AB_1523110 | IHC(1:100) |
| Antibody                          | Rabbit polyclonal anti-Ras | Abcam | Cat# ab180772, RRID:AB_2884935 | WB(1:500) |
| Antibody                          | Mouse monoclonal anti-β-Actin | Sigma-Aldrich | Cat# A1978, RRID:AB_476692 | WB(1:4000) |
| Antibody                          | Rabbit monoclonal anti-Phospho-p44/42 MAPK (Erk1/2) | Cell Signaling Technology | Cat# 4370, RRID:AB_2315112 | WB(1:1000) |
Mice
All animal experiments in this study were performed in accordance with a protocol approved by IACUC of Baylor College of Medicine. In this study, the following mice strains were generated: Arid1a<sup>fl/fl</sup>; Lox-Stop-Lox-Kras<sup>G12D/+</sup>; Ptf1a<sup>CreERT<sup>+</sup></sup>, Arid1a<sup>fl/fl</sup>; Lox-Stop-Lox-Kras<sup>G12D/+</sup>; Ptf1a<sup>CreERT<sup>+</sup></sup>, and Arid1a<sup>+/+</sup>; Lox-Stop-Lox-Kras<sup>G12D/+</sup>; Ptf1a<sup>CreERT<sup>+</sup></sup>. The mouse with the target allele type of Lox-Stop-Lox-Kras<sup>G12D/+</sup> was ordered from Jackson Laboratory (Stock No: 008179 | LSL-K-ras G12D). The promoter of Ptf1a is used to drive the expression of inducible Cre in adult acinar cells (Stock No: 019378 | Ptf1a CreERT<sup>+</sup>, denoted as C). Removal of the floxed exon 8 of the Arid1a (denoted as A<sup>f</sup>) leads to loss of Arid1a transcript (Gao, 2008). Oncogenic Kras mutant allele is silenced by a floxed STOP cassette (Lox-Stop-Lox-Kras<sup>G12D/+</sup>, denoted as K). The adult mice (6–8 weeks) of the above genotypes were administered with tamoxifen (75 mg/kg/day for five consecutive days) to induce efficient ablation of Arid1a and activation of oncogenic Kras in pancreatic acinar cells.

Cell lines
We received the HPNE cell line (RRID:CVCL_C466) from Dr. Jennifer Bailey’s lab (UT Health Center), and the line is cultured in media recommended by ATCC. The identity of the cell line has been authenticated by STR profiling, and the mycoplasma contamination in cell lines has been routinely tested. HPNE with inducible KRAS<sup>G12D/+</sup> (iKRAS- HPNE) was generated by transduction of plasmid pInducer20-KRAS<sup>G12D</sup> into parental HPNE cells. After G418 selection for 15 days, the survived cells were then used for single-cell expansion in 96-well plate. For ARID1A knockout HPNE cells, isogenic clone of iKRAS-HPNE was infected with lentivirus packaged with pl-CRISPR.EFS.tRFP-sgARID1A. After infection for 5 days, the RFP-positive cells were sorted and single-cell expansion was performed. ARID1A knockout was confirmed by Sanger sequencing of the isogenic clones. The guide RNA sequence used for ARID1A knockout is CAGCGGTACCCGATGACCAT. For ALDH1A1 knockdown, two ARID1A knockout HPNE clones were infected by lentivirus packaged with plasmid pGIPz-ALDH1A1. After infection for 5 days, the GFP-positive cells were sorted. Knockdown efficiency was confirmed by RT-PCR. The shRNA sequence targeting ALDH1A1 is GGAGTGTTTACCAAGACATT. For knockdown of C/EBPβ, EP300, or NR3C1, HPNE cells with ARID1A knockout were transfected with plasmid pGIPz-EP300 or pGIPz-NR3C1. After infection for 5 days, the GFP-positive cells were sorted. Knockdown efficiency was confirmed by RT-PCR. The shRNA sequences targeting NR3C1 are CCAACGGTGGCAATGTGAA and AGCTGTAAAGTTTTCTTCA; and targeting EP300 are CGGCAACAGTTGTGCACA and AGCTACTGAAGATAGATTA.

Primary acinar isolation and culture
Pancreata isolated from KC mice and AKC mice 1 month after 5-day tamoxifen administration were rinsed twice in cold 1× HBSS buffer, then minced into small pieces and digested with digestion buffer (HBSS buffer with 10 mM HEPES and 0.5 mg/ml collagenase and 0.25 mg/ml trypsin inhibitor) for 20–30 min at 37°C. During the incubation, the tissue was pipetted every 5 min. After washing twice with washing buffer (HBSS buffer with 5% FBS), the digested tissue was resuspended with media (Waymouth media with 2.5% FBS and 0.25 mg/ml trypsin inhibitor and 100 U/ml Penicillin-Streptomycin), filtrated with 100 µm strainer and seeded into 10 cm dishes overnight at 37°C to remove fibroblasts and ductal cells. The unattached acinar cells were then transferred into collagen-coated plates for growth. To activate Kras expression, 25 ng/ml EGF was added into the media for 5 days. Cells were then used for SA-β-Gal staining, RT-PCR, and western blot.

Western blot
Western blot was performed using the standard protocol. Antibodies used in this study include ALDH1A1 (Abcam Cat# ab23375, RRID:AB_2224009), ALDH3A1 (Abcam Cat# ab76976, RRID:AB_1523110), KRAS (Abcam Cat# ab180772, RRID:AB_2884935), phosph-Erk1/2 (Cell Signaling Technology Cat# 4370, RRID:AB_2315112), and β-actin (Sigma-Aldrich Cat# A1978, RRID:AB_476692).

Colonies formation assay
HPNE cells (3 × 10<sup>4</sup>/well) were seeded into 6-well plates. The cells were treated with doxycycline (6 µg/ml for 15 days) with and without DEAB (1.5 µM for 30 days, Stemcell Technologies Inc 01705).
The media was changed every 2 days. When the colonies were large enough, the cells were fixed and stained with crystal violet.

**ROS measurement**

HPNE cells were treated with doxycycline (6 µg/ml for 5 days). ROS level was measured by Flow Cytometry using ROS Detection Assay Kit (BioVision, Cat# K936-250).

**SA-β-Gal staining**

SA-β-Gal staining was performed on slides of freshly frozen tissues or cells using Senescence β-Galactosidase Staining Kit (Cell Signaling Technology, Cat# 9860). Total and SA-β-Gal-positive lesions or cells were counted at random fields under the microscope, and positive rates were calculated. For quantification of SA-β-Gal staining of primary acinar cells, due to the difficulty of recognizing the nuclei, average optical density (OD) was used to quantify the intensity of SA-β-Gal staining. 8-bit images were adjusted for white balance and color-deconvoluted using Feulgen light green vector in ImageJ. The average gray values of the green channel were measured. OD was calculated using the following formula: $\text{OD} = \log_{10} \left( \frac{255}{\text{gray value}} \right)$.

**PanIN quantification**

Tissues were fixed in 4% paraformaldehyde overnight, processed, and embedded in paraffin. Paraffin-embedded sections were subjected to hematoxylin and eosin staining (H&E staining). ADM, mPanIN-1A, mPanIN-1B, mPanIN-2, and mPanIN-3 were quantified using ImageJ for morphometric analysis based on scanned H&E slides at 20× magnification. Grades of lesions were determined based on the characteristic morphology criterion (Gopinathan et al., 2015) with consulting of pathology core. PanIN-1A: flat epithelium composed of columnar cells with basally oriented nuclei. PanIN-1B: identical to PanIN-1A lesions but exhibit papillary or basally pseudostratified architecture. PanIN-2: show mild nuclear abnormalities, including loss of polarity, nuclear enlargement, nuclear crowding, and nuclear pleomorphism. PanIN-3: show a predominantly papillary or micropapillary architecture with abnormal cribriforming, budding, and luminal necrosis; more severe cytological atypia, such as loss of nuclear polarity, dystrophic goblet cells, nuclear irregularities, and macro nucleoli. In case the lesion harbors more than one PanIN grade, the lesion was graded based on the component with the highest grade. Numbers of lesions of different grades were counted for at least five fields of view. The area of tissue was measured for each field of view. Lymph nodes of the pancreatic area were excluded. Numbers of lesions and tissue areas were summed up to calculate lesion number per area.

**IHC quantification**

For quantification of IHC results against ALDH3A1, H-score method was used. In brief, staining intensity (not stained: 0; weakly stained: +1; moderately stained: +2; or strongly stained: + 3) was determined for each lesion of interest in the field. The H-score was calculated by the following formula: $3 \times \text{percentage of strongly stained cells} + 2 \times \text{percentage of moderately stained cells} + 1 \times \text{weakly stained cells}$, giving a range of 0–300.

**Bulk RNA-seq**

HPNE cells were treated with doxycycline (6 µg/ml) for 5 days. RNA samples were prepared using the standard protocol for Trizol. mRNA was enriched using NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB, E7490), and the library was prepared using the NEBNext Ultra II RNA Library Prep Kit for Illumina (NEB, E7770). All libraries were sequenced on Illumina Nextseq500 platform. Reads were aligned to hg19 assembly of the human genome by STAR aligner (Dobin et al., 2013), and transcripts counting was performed by HTseq-count (Anders et al., 2015). Differential gene expression analysis was performed by using edgeR (Robinson et al., 2010) with a cutoff of FDR at 0.05. To identify the genes with differential response to oncogenic KRAS in KO and WT cells, we also performed the interaction analysis in edgeR.

**Analysis of ALDH1A1 expression in normal pancreas and PDAC**

The expression profiles of ALDH genes in normal pancreas were obtained from GTEx database. The expression level of ALDH1A1 in different cell types in normal pancreas was obtained from Human
Protein Atlas database. The PDAC RNA-seq data were from ICGC-PACA-AU cohort. The raw count data were downloaded from https://dcc.icgc.org/.

**ATAC-seq experiment**

ATAC-seq was performed following the protocol of Howard Chang’s lab (https://www.nature.com/articles/nmeth.4396) with slight modifications. In brief, 5 × 10⁴ cells were lysed with ATAC-Resuspension Buffer (RSB) containing 0.1% NP40 and 0.1% Tween-20. After incubation on ice for 3 min, the cell lysates were washed by RSB with 0.1% Tween-20. The cell lysates were then incubated with transposition mixture at 37°C for 30 min. After amplification, the transposed fragments were purified with magnetic beads. Finally, 4 ng fragments were used for the generation of the library. All libraries were sequenced on Illumina Nextseq500 platform.

**ATAC-seq data analysis**

Reads were then mapped to the hg19 assembly by Bowtie2 (Langmead and Salzberg, 2012) after removing the adaptor sequence. The quality control of ATAC-seq data was performed by using the ATACseqQC R package (Ou et al., 2018). Next, the mapped reads from three technical replicates of each genotype were combined for the peak calling by MACS2 (Zhang et al., 2008). Peaks from wildtype samples and ARID1A-KO samples were combined to get a union peak set. All the peaks were then annotated by HOMER (Heinz et al., 2010). HTseq-count (Anders et al., 2015) was used for read counting. edgeR package (Robinson et al., 2010) was then used for normalization between different samples and for peak differential analysis. The read density profiles of the differential peaks were plotted by deepTools (Ramírez et al., 2016). The motif enrichment analysis was performed by using AME.

**ENCODE data analysis**

The expression levels of ALDH1A1 in the cell lines in ENCODE were obtained from the Cancer Cell Line Encyclopedia (CCLE) database (file name: file-CCLE_RNAseq_genes_rpmk_20180929.gct.gz; Ghandi et al., 2019). Seven cell lines with RPKM >10 were categorized as ALDH1A1high cell lines. Five cell lines with RPKM <0.5 were selected as ALDH1A1low cell lines. The A549 H3K27ac and H3K4me1 ChIP-seq data were reanalyzed by using hg19 assembly as described above. The peak files of H3K27ac/H3K4me1 from the other cell lines were directly obtained from the ENCODE database. The ALDH1A1-related differential ATAC peaks, which are overlapped with the H3K27ac/H3K4me1 peaks in at least four out of seven ALDH1A1high cell lines, were characterized as functional enhancer regions.

**PanIN-seq**

We used the MMI CellCut platform to perform LCM. 40–50% laser power was used with the cutting speed of 18 µm/s to dissect microscopic lesions. 1.6 µl of MATQ-seq lysis buffer (1 µl of 0.2% Triton X100 [Sigma-Aldrich], 0.4 µl of primer mix, 0.12 µl dNTP, 0.05 µl 0.1 M DTT [Life Technologies], and 2 U RNaseOUT [Life Technologies]) was added onto the isolation cap where the dissected tissue was attached (MMI, Prod. No. 50206). We used a pipette tip to scrape the laser-dissected tissue into the lysis buffer and then pipetted the lysis buffer into the tube. Sample tubes were then placed on a thermocycler and incubated at 72°C for 3.5 min, followed by 1 min incubation on ice. 2.4 µl of MATQ-seq first strand synthesis buffer (0.8 µl 5× First Strand Buffer [Life Technologies], 0.2 µl 0.1 M DTT, 4 U RNaseOUT [Life Technologies], 30 U Superscript III [Life Technologies], and 1.15 µl RNase-free water) was then added. The reverse transcription program was same as MATQ-seq. After reverse transcription, the residual primers were then digested by using T4 polymerase at 37° for 40 min and 75° for 20 min. RNA was then digested by using RNase-H and RNase-If at 37° for 15 min and 72° for 15 min. Following that, dC-tailing and second strand synthesis were performed as described in MATQ-seq. The library prep for PanIN-seq samples were same as MATQ-seq. All libraries were sequenced on Illumina Nextseq500 platform.

**PanIN-seq data analysis**

The raw sequencing data trimming and barcode retrieval were performed as previously described (Sheng et al., 2017). The reads were mapped to the genome MM10 using STAR with the
following parameters: --outFilterMismatchNoverLmax 0.05 --outFilterMatchNmin 16
--outFilterScoreMinOverLread 0 --outFilterMatchNminOverLread 0. We used Gencode
annotation release mm10 (GRCm38.p4) for transcript annotation. Unique barcode counting and gene
expression-level quantification were performed as previously described with a few modifications: the
mapping position of the reads was included as part of the identity of the corresponding barcodes;
only reads mapped to the exon region were used for gene expression-level quantification. The genes
with APM >2 in at least five samples were retained for differential gene expression analysis, which was
performed by using edgeR with a cutoff of FDR at 0.05.

**Functional enrichment analysis**

Functional enrichment analysis was performed by using GSEA with the MSigDB Hallmark gene sets,
and the senescence-related gene sets from the MSigDB curated gene sets. For PanIN-seq, the permu-
tation of phenotype label was used to calculate the p-value.

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**Author contributions**

Shou Liu, Data curation, Formal analysis, Investigation, Methodology, validation, visualization, Writing
– original draft, Writing – review and editing; Wenjian Cao, Yichi Niu, Data curation, Formal analysis,
Investigation, Methodology, Writing – original draft, Writing – review and editing; Jiayi Luo, Investiga-
tion; Yanhua Zhao, Zhiying Hu, Project administration; Chenghang Zong, Conceptualization, Funding
acquisition, Investigation, Methodology, Project administration, Supervision, Writing – original draft,
Writing – review and editing

**Author ORCIDs**

Yichi Niu [i](http://orcid.org/0000-0002-4376-7792)
Jiayi Luo [i](http://orcid.org/0000-0002-9873-0671)
Chenghang Zong [i](http://orcid.org/0000-0002-8337-8038)

**Ethics**

This study was performed in strict accordance with the recommendations in the Guide for the Care
and Use of Laboratory Animals of the National Institutes of Health. All of the animals were handled
according to approved institutional animal care and use committee (IACUC) protocols (#AN-6434) of
Baylor College Medicine. Every effort was made to minimize suffering.

**Decision letter and Author response**

Decision letter [i](https://doi.org/10.7554/eLife.64204.sa1)
Author response [i](https://doi.org/10.7554/eLife.64204.sa2)
Additional files

Supplementary files
- Supplementary file 1. Gene set enrichment analysis on PanIN-seq.
- Supplementary file 2. Differential gene expression analysis on PanIN-seq.
- Supplementary file 3. Differential gene expression analysis on RNA-seq.
- Supplementary file 4. Interaction test on the RNA-seq data.
- Supplementary file 5. Quality control of ATAC-seq data.
- Supplementary file 6. ATAC peaks in the regulatory regions of ALDH1A1.
- Supplementary file 7. Motif enrichment analysis on differential ATAC peaks.
- Supplementary file 8. Expression (RPKM) of ALDH1A1 in other human cell lines.
- Supplementary file 9. Binding of TFs at the regulatory region of ALDH1A1 in human cell lines.
- Supplementary file 10. List of ENCODE datasets used.
- Transparent reporting form

Data availability
Sequencing data have been deposited in GEO under GSE160444.

The following dataset was generated:

| Author(s) | Year | Dataset title | Dataset URL | Database and Identifier |
|-----------|------|---------------|-------------|-------------------------|
| Liu S, Cao WJ, Niu YC, Luo JY, Zhao JH, Zong C, Hu Z | 2020 | | https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE160444 | NCBI Gene Expression Omnibus, GSE160444 |

The following previously published datasets were used:

| Author(s) | Year | Dataset title | Dataset URL | Database and Identifier |
|-----------|------|---------------|-------------|-------------------------|
| The International Cancer Genome Consortium | 2016 | | https://www.ebi.ac.uk/ega/studies/EGAS00001000154 | European Genome-phenome Archive (EGA), EGAS00001000154 |
| The Cancer Genome Atlas Research Network | 2017 | | https://portal.gdc.cancer.gov/projects/TCGA-PAAD | GDC Data Portal, TCGA-PAAD |

References

Anders S, Pyl PT, Huber W. 2015. HTSeq--a Python framework to work with high-throughput sequencing data. Bioinformatics 31: 166–169. DOI: https://doi.org/10.1093/bioinformatics/btu638, PMID: 25260700

Bailey P, Chang DK, Nones K, Johns AL, Patch A-M, Gingras M-C, Miller DK, Christ AN, Bruxner TJ, Quinn MC, Nourse C, Murtaugh LC, Harliwong I, Idrisoglu S, Manning S, Nourbakhsh E, Wani S, Fink L, Holmes O, Chin V, et al. 2016. Genomic analyses identify molecular subtypes of pancreatic cancer. Nature 531: 47–52. DOI: https://doi.org/10.1038/nature10600, PMID: 26909576

Baker DJ, Wijshake T, Tchkonia T, LeBrasseur NK, Childs BG, van de Sluis B, Kirkland JL, van Deursen JM. 2011. Clearance of p16ink4a-positive senescent cells delays ageing-associated disorders. Nature 479: 232–236. DOI: https://doi.org/10.1038/nature10600, PMID: 22048312

Cancer Genome Atlas Research Network, Cancer Genome Atlas Research Network. 2017. Integrated genomic characterization of pancreatic ductal adenocarcinoma. Cancer Cell 32: 185-203. DOI: https://doi.org/10.1016/j.ccell.2017.07.007, PMID: 28810144

DeNicola GM, Karreth FA, Humphton TJ, Gopinathan A, Wei C, Frese K, Mangal D, Yu KH, Yeo CJ, Galhoun ES, Scrimieri F, Winter JM, Hruban RH, Iacobuzio-Donahue C, Kern SE, Blair IA, Tuveson DA. 2011. Oncogene-induced NRF2 transcription promotes ROS detoxification and tumorigenesis. Nature 475: 106–109. DOI: https://doi.org/10.1038/nature10189, PMID: 21734707

Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, Medrano EE, Linkens M, Rubelj I, Pereira-Smith O. 1995. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. PNAS 92: 9363–9367. DOI: https://doi.org/10.1073/pnas.92.20.9363, PMID: 7568133
Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR. 2013. Star: Ultrafast Universal RNA-SEQ aligner. Bioinformatics 29: 15–21. DOI: https://doi.org/10.1093/bioinformatics/bts635, PMID: 23104886

ENCODE Project Consortium. 2012. An integrated Encyclopedia of DNA elements in the human genome. Nature 489: 57–74. DOI: https://doi.org/10.1038/nature11247, PMID: 22955616

Gao X. 2008. ES cell pluripotency and germ-layer formation require the SWI/SNF chromatin remodeling component BAF250a. PNAS 105: 6656–6661. DOI: https://doi.org/10.1073/pnas.0801802105, PMID: 18446678

Ghandi M, Huang FW, Jané-Valbuena J, Kryukov GV, Lo CC, McDonald ER, Barretina J, Gelfand ET, Bielski CM, Li H, Hu K, Andreev-Draklin AY, Kim J, Hess JM, Haas BJ, Aguet F, Weir BA, Rotheberg MV, Paolella BR, Lawrence MS, et al. 2019. Next-generation characterization of the cancer cell line encyclopedia. Nature 569: 503–508. DOI: https://doi.org/10.1038/s41586-019-1186-3, PMID: 31068700

Gopinathan A, Huang FW, Jané-Valbuena J, Kryukov GV, Lo CC, McDonald ER, Barretina J, Gelfand ET, Bielski CM, Ghandi M, Hargraves DC, Hodges C, Cheng JX, Murre C, Singh H, Glass CK. 2010. Simple post- alignment quality assessment of Atac-seq data. 35: 177-190. DOI: https://doi.org/10.1016/j.ccell.2018.12.009, PMID: 30686770

Ikuta K, Yoshioka T, Wang Z, Akiyama H, Wright CV, Takaori K, Uemoto S, Chiba T, Seno H. 2018. ARID1A Maintains Differentiation of Pancreatic Ductal Cells and Inhibits Development of Pancreatic Ductal Adenocarcinoma in Mice. Gastroenterology 155: 194-209. DOI: https://doi.org/10.1053/j.gastro.2018.03.039, PMID: 29604291

Livshits G, Alonso-Curbelo D, Morris JP, Coche R, Saborowski M, Wilkinson JE, Lowe SW. 2018. ARID1A loss impairs enhancer-mediated gene regulation and drives colon cancer in mice. Nature Genetics 50: 592–601. DOI: https://doi.org/10.1038/ng.2628, PMID: 23644491

McLean CY, Bristol D, Hiller M, Clarke SL, Schaar BT, Lowe CB, Wenger AM, Bejerano G. 2010. Great improves functional interpretation of cis-regulatory regions. Nature Biotechnology 28: 495–501. DOI: https://doi.org/10.1038/nbt.1630, PMID: 20436461

MeLeay RC, Bailey TL. 2010. Motif Enrichment Analysis: a unified framework and an evaluation on ChIP data. BMC Bioinformatics 11: 165. DOI: https://doi.org/10.1186/1471-2105-11-165, PMID: 20356413

Mei S, Qin Q, Wu Q, Sun H, Zheng R, Zang C, Zhu M, Wu J, Shi X, Taing L, Liu T, Brown M, Meyer CA, Liu XS. 2017. Cistrome data browser: A data portal for chip-seq and chromatin accessibility data in human and mouse. Nucleic Acids Research 45: D658–D662. DOI: https://doi.org/10.1093/nar/gkw983, PMID: 27789702

Morton JP, Timpson P, Karim SA, Ridgway RA, Athineos D, Doyle B, Jamieson NB, Oien KA, Lowy AM, Brunton VG, Frame MC, Evans TRJ, Sansom OJ. 2010. Mutant p53 drives metastasis and overcomes growth arrest/senescence in pancreatic cancer. PNAS 107: 246–251. DOI: https://doi.org/10.1073/pnas.0908428107, PMID: 20018721

Ogiwara H, Takahashi K, Sasaki M, Kuroda T, Yoshida H, Watanabe R, Maruyama A, Makinoshima H, Chiwaki F, Sasaki H, Kato T, Okamoto A, Kohno T. 2019. Targeting the vulnerability of glutathione metabolism in arid1a-deficient cancers. Cancer Cell 35: 177-190. DOI: https://doi.org/10.1016/j.ccell.2018.12.009, PMID: 30686770

Pignolo RJ, Passos JF, Khosla S, Tchkonia T, Kirkland JL. 2020. Reducing senescent cell burden in aging and disease. Trends in Molecular Medicine 26: 630–638. DOI: https://doi.org/10.1016/j.molmed.2020.03.005, PMID: 32589933

Raha D, Wilson TR, Peng J, Peterson D, Yue P, Evangelista M, Wilson C, Merchant M, Settleman J. 2014. The cancer stem cell marker aldehyde dehydrogenase is required to maintain a drug-tolerant tumor cell subpopulation. Cancer Research 74: 3579–3590. DOI: https://doi.org/10.1158/0008-5472.CAN-13-3456, PMID: 24812274
Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. 2014. Projecting cancer incidence and deaths to 2030: The unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Research 74: 2913–2921. DOI: https://doi.org/10.1158/0008-5472.CAN-14-0155, PMID: 24840647

Ramirez F, Ryan DP, Grüning B, Bhardwaj V, Kilpert F, Richter AS, Heyne S, Dünnar F, Manke T. 2016. DeepTools2: A next generation web server for deep-sequencing data analysis. Nucleic Acids Research 44: W160-W165. DOI: https://doi.org/10.1093/nar/gkw257, PMID: 27079975

Robinson MD, McCarthy DJ, Smyth GK. 2010. Edger: A bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26: 139–140. DOI: https://doi.org/10.1093/bioinformatics/btp616, PMID: 19910308

Rovira M, Scott S-G, Liss AS, Jensen J, Thayer SP, Leach SD. 2010. Isolation and characterization of centroacinar/terminal ductal progenitor cells in adult mouse pancreas. PNAS 107: 75–80. DOI: https://doi.org/10.1073/pnas.0912589107, PMID: 20018761

Sen M, Wang X, Hamdan FH, Rapp J, Eggert J, Kosinsky RL, Wegwitz F, Kutschkat AP, Younges FS, Gaedcke J, Grade M, Henschke E, Papantoni A, Strübel P, Johnsen SA. 2019. ARID1A facilitates KRAS signaling-regulated enhancer activity in an AP1-dependent manner in colorectal cancer cells. Clinical Epigenetics 11: 92. DOI: https://doi.org/10.1186/s13148-019-0690-5, PMID: 31217031

Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW. 1997. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4A. Cell 88: 593–602. DOI: https://doi.org/10.1016/s0092-8674(00)81902-9, PMID: 9054499

Sheng K, Cao W, Niu Y, Deng Q, Zong C. 2017. Effective detection of variation in single-cell transcriptomes using Matq-seq. Nature Methods 14: 267–270. DOI: https://doi.org/10.1038/nmeth.4145, PMID: 28092691

Son J, Lysyiotis CA, Ying H, Wang X, Hua S, Ligorio M, Perera RM, Ferrone CR, Mullarky E, Shyh-Chang N, Kang Y, Fleming JB, Bardeesy N, Asara JM, Haigis MC, DePinho RA, Cantley LC, Kimmelman AC. 2013. Glutamine supports pancreatic cancer growth through a kras-regulated metabolic pathway. Nature 496: 101–105. DOI: https://doi.org/10.1038/nature12040, PMID: 23535601

Storz P. 2017. KRas, ROS and the initiation of pancreatic cancer. Small GTPases 8: 38–42. DOI: https://doi.org/10.1080/21541248.2016.1192714, PMID: 27215184

Sun X, Wang SC, Wei Y, Luo X, Jia Y, Li L, Gopal P, Zhu M, Nassour I, Chuang JC, Maples T, Celen C, Nguyen LH, Wu L, Fu S, Li W, Hui L, Tian F, Ji Y, Zhang S, et al. 2017. Arid1a has context-dependent oncogenic and tumor suppressor functions in liver cancer. Cancer Cell 32: 574-589.. DOI: https://doi.org/10.1016/j.ccell.2017.10.007, PMID: 29136504

Waddell N, Pajic M, Patch A-M, Chang DK, Kassahn KS, Bailey P, Johns AL, Miller D, Nones K, Quek K, Quinn MCJ, Robertson AJ, Fadlullah MZH, Bruxner TJ, Christ AN, Harlwong I, Idrisoglu S, Manning S, Nourse C, Nourbakhsh E, et al. 2015. Whole genomes redefine the mutational landscape of pancreatic cancer. Nature 518: 495–501. DOI: https://doi.org/10.1038/nature14169, PMID: 25719666

Wang SC, Nassour I, Xiao S, Zhang S, Luo X, Lee J, Li L, Sun X, Nguyen LH, Chuang J-C, Peng L, Daigle S, Shen J, Zhu H. 2019. SWI/SNF component ARID1A restrains pancreatic neoplasia formation. Gut 68: 1259–1270. DOI: https://doi.org/10.1136/gutjnl-2017-315490, PMID: 30315093

Wu JN, Roberts CW. 2013. ARID1A mutations in cancer: another epigenetic tumor suppressor. Cancer Discovery 3: 35–43. DOI: https://doi.org/10.1158/2159-8290.CD-12-0361, PMID: 23208470

Wu S, Fatkhutdinov N, Zhang R. 2017. Harnessing mutual exclusivity between TP53 and ARID1 A mutations. Cell Cycle 2313–2314. DOI: https://doi.org/10.1080/15384101.2017.1377503, PMID: 29279027

Zhang Y, Liu T, Meyer CA, Eeckhoute J, Johnson DS, Bernstein BE, Nusbaum C, Myers RM, Brown M, Li W, Liu XS. 2008. Model-based analysis of ChiP-Seq (MACS). Genome Biology 9:R137. DOI: https://doi.org/10.1186/gb-2008-9-9-r137, PMID: 18798982

Zhang L. 2018. Loss of ARID1A Expression Correlates With Tumor Differentiation and Tumor Progression Stage in Pancreatic Ductal Adenocarcinoma. Technology in Cancer Research & Treatment 17: 1533034618754475. DOI: https://doi.org/10.1177/1533034618754475, PMID: 29486633