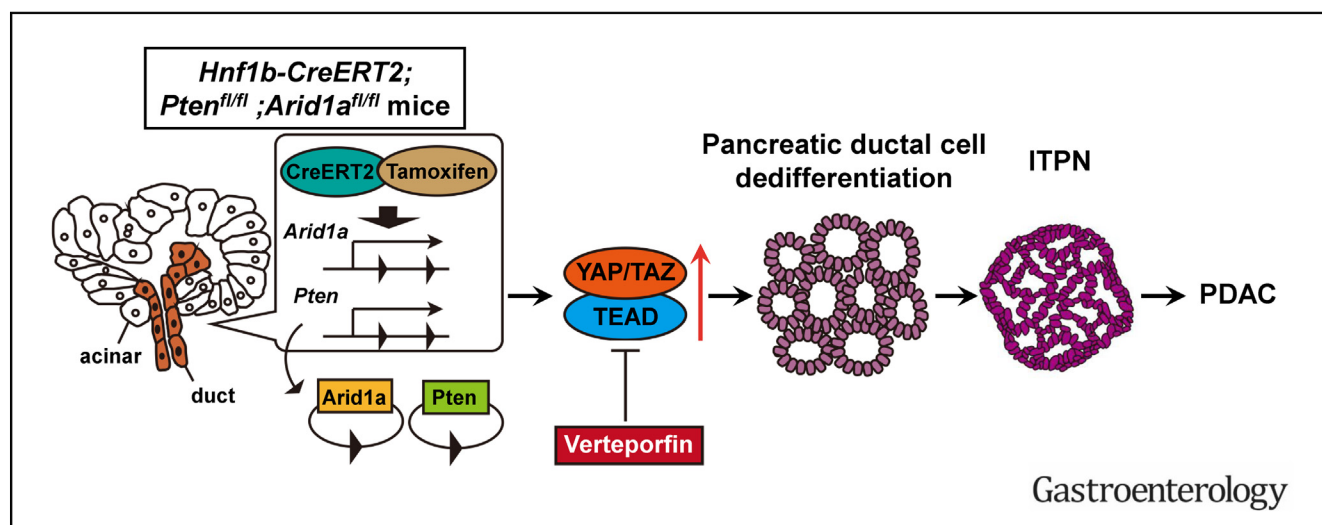


Loss of *Arid1a* and *Pten* in Pancreatic Ductal Cells Induces Intraductal Tubulopapillary Neoplasm via the YAP/TAZ Pathway



Yuichi Fukunaga,^{1,2,3} Akihisa Fukuda,¹ Mayuki Omatsu,¹ Mio Namikawa,¹ Makoto Sono,¹ Tomonori Masuda,¹ Osamu Araki,¹ Munemasa Nagao,¹ Takaaki Yoshikawa,¹ Satoshi Ogawa,¹ Yukiko Hiramatsu,¹ Yu Muta,¹ Motoyuki Tsuda,¹ Takahisa Maruno,¹ Yuki Nakanishi,¹ Jorge Ferrer,⁴ Tatsuaki Tsuruyama,⁵ Toshihiko Masui,⁶ Etsuro Hatano,⁶ and Hiroshi Seno¹

¹Department of Gastroenterology and Hepatology, Kyoto University Graduate School of Medicine, Kyoto, Japan; ²Department of Drug Discovery Medicine, Medical Innovation Center, Kyoto University Graduate School of Medicine, Kyoto, Japan; ³DSP Cancer Institute, Sumitomo Dainippon Pharma Co., Osaka, Japan; ⁴Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona, Spain; ⁵Kyoto University Clinical Bio Resource Center, Kyoto, Japan; and ⁶Division of Hepato-Biliary-Pancreatic Surgery and Transplantation, Department of Surgery, Kyoto University Graduate School of Medicine, Kyoto, Japan



See editorial on page 371.

BACKGROUND & AIMS: Pancreatic ductal adenocarcinoma (PDAC) arises from several types of premalignant lesions, including intraductal tubulopapillary neoplasm (ITPN); however, the molecular pathogenesis of ITPN remains unknown. **METHODS:** We performed studies with *Hnf1b-Cre^{ERT2}; Pten^{fl/fl}; Arid1a^{fl/fl}* mice to investigate the consequence of genetic deletion of *Arid1a* in adult pancreatic ductal cells in the context of oncogenic PI3K/Akt pathway activation. **RESULTS:** Simultaneous deletion of *Arid1a* and *Pten* in pancreatic ductal cells resulted in the development of ITPN, which progressed to PDAC, in mice. Simultaneous loss of *Arid1a* and *Pten* induced dedifferentiation of pancreatic ductal cells and Yes-associated protein 1/Transcriptional coactivator with PDZ-binding motif (YAP/TAZ) pathway activation. Consistent with the mouse data, TAZ expression was found elevated in human ITPNs and ITPN-derived PDACs but not in human intraductal papillary mucinous neoplasms, indicating that activation of the TAZ pathway is a distinctive feature of ITPN. Furthermore, pharmacological inhibition of the YAP/TAZ pathway suppressed the dedifferentiation of pancreatic ductal cells and development of

ITPN in *Arid1a* and *Pten* double-knockout mice. **CONCLUSION:** Concurrent loss of *Arid1a* and *Pten* in adult pancreatic ductal cells induced ITPN and ITPN-derived PDAC in mice through aberrant activation of the YAP/TAZ pathway, and inhibition of the YAP/TAZ pathway prevented the development of ITPN. These findings provide novel insights into the pathogenesis of ITPN-derived PDAC and highlight the YAP/TAZ pathway as a potential therapeutic target.

Keywords: Pancreatic Cancer; SWI/SNF Complex; Mouse Models; YAP/TAZ Pathway; ITPN.

Abbreviations used in this paper: ATAC-seq, assay for transposase-accessible chromatin using sequencing; EMT, epithelial to mesenchymal transition; GSEA, Gene Set Enrichment Analysis; IHC, immunohistochemistry; IPMN, intraductal pancreatic mucinous neoplasm; ITPN, intraductal tubulopapillary neoplasm; pAkt, phosphorylated-Akt; PanIN, pancreatic intraepithelial neoplasia; PDAC, pancreatic ductal adenocarcinoma; PTEN, phosphatase and tensin homologue; qRT-PCR, quantitative reverse-transcriptase polymerase chain reaction; RNA-seq, RNA sequencing; TAZ, Transcriptional coactivator with PDZ-binding motif; VP, verteporfin; YAP, Yes-associated protein 1.

Most current article

© 2022 by the AGA Institute.
0016-5085/\$36.00

<https://doi.org/10.1053/j.gastro.2022.04.020>

Pancreatic ductal adenocarcinoma (PDAC) is a lethal disease with an extremely poor prognosis,¹ and thus early diagnosis and more effective therapies are urgently needed to improve patient outcomes. To this end, a better understanding of the molecular mechanisms underlying PDAC development is crucial. PDAC is thought to arise from premalignant lesions, including pancreatic intraepithelial neoplasia (PanIN), intraductal pancreatic mucinous neoplasm (IPMN), and intraductal tubulopapillary neoplasm (ITPN). ITPN, a rare subset of epithelial neoplasms of the pancreas representing approximately 3% of premalignant pancreatic tumors and 0.9% of all pancreatic exocrine neoplasms,² is characterized by intraductal tubulopapillary growth and cellular dysplasia, and has been recently defined as a distinct entity in the World Health Organization classification based on its unique morphological and genomic features.²⁻⁴ The morphological and histological features of ITPNs that distinguish them from IPMNs include no observable secreted mucin, cribriform structure, tubulopapillary growth, cuboidal cells with little cytoplasmic mucin, uniform high-grade atypia, and no MUC2 and MUC5AC expression.⁴ Most ITPNs grow in the main pancreatic duct with upstream dilatation and mimic PDAC, and those growing only in branch ducts are extremely rare.

Recent sequencing studies have revealed that activating mutations in *KRAS* are common in human PanINs⁵ and PDACs⁶ and are often observed in IPMNs^{7,8}; however, these mutations are rarely found in patients with ITPN.^{2,9} Approximately 30% of ITPNs have activating mutations in genes involved in the PI3K/Akt pathway (*PIK3CA*, *PIK3CB*, *INPP4A*, *PTEN*) and chromatin-remodeling factors (*MLL1*, *MLL2*, *MLL3*, *BAP1*, *PBRM1*, *EED*, and *ATRX*), suggesting that ITPN harbors distinct genetic alterations.⁹ However, the molecular mechanisms underlying ITPN and ITPN-derived PDAC development are largely unknown, and genetically engineered mouse models of ITPN and ITPN-derived PDAC have not yet been established.

Recent genome-wide sequencing studies have shown that mutations in subunit genes of the SWI/SNF chromatin-remodeling complexes are widespread across diverse human cancers^{10,11} and have been found in 12% to 23% of human PDACs.^{6,10,12} SWI/SNF complexes remodel the chromatin structure using energy from ATP hydrolysis to modulate gene expression.¹³ *ARID1A* is the most frequently mutated subunit in SWI/SNF complexes in human PDAC,¹⁴ and decreased *ARID1A* protein expression is found in various types of cancers, including PDAC.¹⁵⁻¹⁷ *ARID1A* plays a role in the specific recruitment of the SWI/SNF complex by binding to transcription factors.¹⁸ Using a mouse model, we recently showed that loss of *Arid1a* in pancreatic ductal cells synergistically acts with oncogenic *Kras* to induce dedifferentiation of pancreatic ductal cells and IPMN development, which subsequently develops into invasive PDAC.¹⁹ Furthermore, another study showed that loss of *Arid1a* in the context of oncogenic *Kras* led to the development of IPMN as well as the activation of epithelial to mesenchymal transition (EMT) and stem cell transcriptional programs in *Arid1a*-deficient murine PDAC cells.²⁰

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

Intraductal tubulopapillary neoplasm has unique mutation features, including no *KRAS* mutation and frequent mutation in chromatin-remodeling factors and the PI3K/AKT pathway; however, the molecular mechanism of intraductal tubulopapillary neoplasm development is largely unknown.

NEW FINDINGS

Simultaneous loss of *ARID1A* and phosphatase and tensin homologue in pancreatic ductal cells induces development of intraductal tubulopapillary neoplasm through dedifferentiation and aberrant activation of the Yes-associated protein 1/Transcriptional coactivator with PDZ-binding motif (YAP/TAZ) pathway, and pharmacological inhibition of the YAP/TAZ pathway suppresses intraductal tubulopapillary neoplasm formation in mice.

LIMITATIONS

Further studies are required to elucidate the molecular mechanism of aberrant activation of the YAP/TAZ pathway in *ARID1A*- and *PTEN*-mutated pancreatic ductal cells.

IMPACT

Aberrant activation of the YAP/TAZ pathway is a molecular feature of intraductal tubulopapillary neoplasm and intraductal tubulopapillary neoplasm-derived pancreatic ductal adenocarcinomas, and inhibition of the YAP/TAZ pathway could be a novel therapeutic approach for intraductal tubulopapillary neoplasm and intraductal tubulopapillary neoplasm-derived pancreatic ductal adenocarcinomas.

Recent studies have reported a high co-occurrence of *ARID1A* with *PTEN* or *PIK3CA* mutations in several types of cancer, including ovarian clear cell carcinoma, uterine endometrioid carcinoma, and uterine serous carcinoma.^{21,22} These findings suggest a synergistic effect between the loss of *Arid1a* and activation of the PI3K/Akt pathway on oncogenesis. However, the *in vivo* role of *ARID1A* in pancreatic tumorigenesis in the context of oncogenic activation of the PI3K/Akt pathway remains unknown.

Therefore, in this study, we aimed to investigate the impact of *Arid1a* inactivation in pancreatic ductal cells in the context of oncogenic PI3K/Akt pathway activation and clarify the functional role of *Arid1a* in the specification and development of PDAC and its precursors in mice.

Materials and Methods

Mice

Experimental animals were generated by crossing *Hnf1b-CreERT2* mice (a gift from Jorge Ferrer, Imperial College, London, UK),²³ *Arid1a*^{flox} mice,²⁴ and *Pten*^{flox} mice (Stock no. 004597; The Jackson Laboratory, Bar Harbor, ME). The mice were crossed with a mixed background. For the induction of Cre-mediated recombination, tamoxifen (Sigma-Aldrich, St Louis, MO) was administered a total of 3 times: once a day by

oral gavage at a dose of 400 mg/kg, every 2 days. Pancreatic tissue from *Ptf1α-Cre; Kras^{G12D}; Arid1a^{flox/flox}* mice were prepared as described previously.¹⁹ All experiments involving mice were approved by the animal research committee of Kyoto University (Kyoto, Japan) and performed in accordance with Japanese government regulations.

Histology and Immunohistochemistry

For histological analyses, mouse organs were isolated and fixed overnight in 4% paraformaldehyde, embedded in paraffin, and sectioned at a thickness of 5 μm. Paraffin-embedded sections were stained with hematoxylin and eosin. We classified pancreatic lesions using the same diagnostic criteria used for humans.²⁵ For immunohistochemistry (IHC), antigen retrieval was performed by boiling the sections in 10 mM citric acid buffer (pH 6.0) or EDTA buffer (pH 8.0) for 15 minutes at 98°C. The samples were then incubated with primary antibodies overnight at 4°C in a humidified chamber, followed by secondary antibody incubation for 1 hour at room temperature. Peroxidase-streptavidin labeling was performed using the VECTASTAIN Elite ABC Standard Kit (Cat. no. PK-6100; Vector Laboratories, Burlingame, CA). The sections were then stained with diaminobenzidine substrate (Cat. no. K3468; Dako, Carpinteria, CA) and counterstained with hematoxylin (Cat. no. 109249; Sigma-Aldrich).

Clinical Samples

Surgically resected specimens of 5 human ITPNs and 21 human IPMNs were obtained from patients admitted to Kyoto University Hospital. The study protocol was approved by the Ethics Committee of the Kyoto University Hospital (R2904).

Statistics

Data are presented as the mean ± SD. Statistical comparisons between groups were performed using 1-way analysis of variance with Dunnett's test or Tukey's test and Mann-Whitney test. *P* values < .05 were considered statistically significant. All statistical analyses were performed using GraphPad Prism (version 7.03; GraphPad Software Inc., La Jolla, CA) or JMP Pro 15 (SAS Institute Inc., Cary, NC).

Data Availability

The RNA sequencing (RNA-seq) and assay for transposase-accessible chromatin using sequencing (ATAC-seq) data will be deposited in the Gene Expression Omnibus database before publication.

Detailed methods are described in the [Supplementary Material and Methods](#). Primary antibodies for IHC are listed in [Supplementary Table 1](#). Primary antibodies for Western blot are listed in [Supplementary Table 2](#). Primer sequences are described in [Supplementary Table 3](#).

Results

Simultaneous Loss of *Arid1a* and *Pten* in Pancreatic Ductal Cells Drives the Formation of ITPN and ITPN-derived PDAC in Mice

To investigate the functional role of *Pten* and *Arid1a* in murine pancreatic ductal cells, we crossed *Hnf1b-Cre^{ERT2}*

mice, which express tamoxifen-inducible Cre in adult pancreatic ductal cells ([Figure 1A](#)),²³ with *Pten^{ff}* and *Arid1a^{ff}* mice and established 4 murine models, designated as *H* (*Hnf1b-Cre^{ERT2}*), *HA* (*Hnf1b-Cre^{ERT2}; Arid1a^{ff}*), *HP* (*Hnf1b-Cre^{ERT2}; Pten^{ff}*), and *HPA* (*Hnf1b-Cre^{ERT2}; Pten^{ff}; Arid1a^{ff}*) mice.

H, *HA*, *HP*, and *HPA* mice were histologically examined 12 weeks after oral administration of tamoxifen ([Figure 1B](#)); the *H* and *HA* mouse pancreas showed no microscopic abnormalities, whereas mild dysplasia of the main pancreatic duct only in the head of the pancreas without dilation and no dysplasia in the branch ducts was observed in *HP* mice ([Figure 1C](#)). In contrast, the dual loss of *Arid1a* and *Pten* in pancreatic ductal cells resulted in the development of pancreatic intraductal neoplasms in *HPA* mice ([Figure 1C](#)). Dense chromatin and mitotic figures were also identified in the pancreatic tissues of *HPA* mice. These neoplastic lesions grew in a tubular structure in the main pancreatic duct with upstream dilation as well as in the branch ducts. The tumor cells were eosinophilic, cuboidal, had round to oval-shaped nuclei, and were arranged back-to-back in complex tubular structures that were predominantly cribriform with focal papillary formation. These histological findings are consistent with the typical features of human ITPN.^{2,3,9}

We further observed elevated Ki67 expression in *HPA* mouse ITPN-like lesions compared with that in *HP* or *HA* mouse pancreatic ductal cells, suggesting that ITPN tumor cells in *HPA* mice are actively proliferating ([Figure 1D](#)). The Cre-based recombination in pancreatic ductal cells was robust, as confirmed by IHC showing a lack of *Arid1a* expression in *HA* mice and both decreased expression of *Pten* and positive expression of phosphorylated-Akt (pAkt) in pancreatic ductal cells in *HP* mice ([Figure 1D](#) and [Supplementary Figure 1A](#)).

Human ITPNs are characterized by mucin glycoprotein expression that is usually positive for MUC1 but negative for MUC2 and MUC5AC, whereas pancreatobiliary-type human and mouse IPMNs are positive for MUC1 and MUC5AC but negative for MUC2.^{3,9} Therefore, we performed IHC for these mucin glycoproteins in ITPN-like lesions from *HPA* mice. Pancreatic intraductal neoplastic cells in *HPA* mice were positive for MUC1 but negative for MUC2 and MUC5AC and pancreatic ductal cells in *H*, *HA*, and *HP* mice represented the same expression pattern ([Figure 1E](#), [Supplementary Figure 1B](#)). This indicates that the lesions closely resemble human ITPNs not only in morphology but also in terms of mucin expression patterns.

We next analyzed the 4 mouse genotypes at different time points and found no abnormalities in *H* and *HA* mice at 52 weeks after tamoxifen administration and no neoplastic lesions in *HP* mice even at 52 weeks after tamoxifen administration ([Figure 2A](#)). Although no apparent abnormality was observed at 4 weeks, ITPN-like lesions developed in all *HPA* mice 8 weeks after tamoxifen administration with 100% penetrance. In addition, some of these lesions had an extraductal microinvasion of 0.05 mm in depth and desmoplasia surrounding tumor cells, mimicking PDAC with microinvasion ([Figure 2B](#)). *HPA*

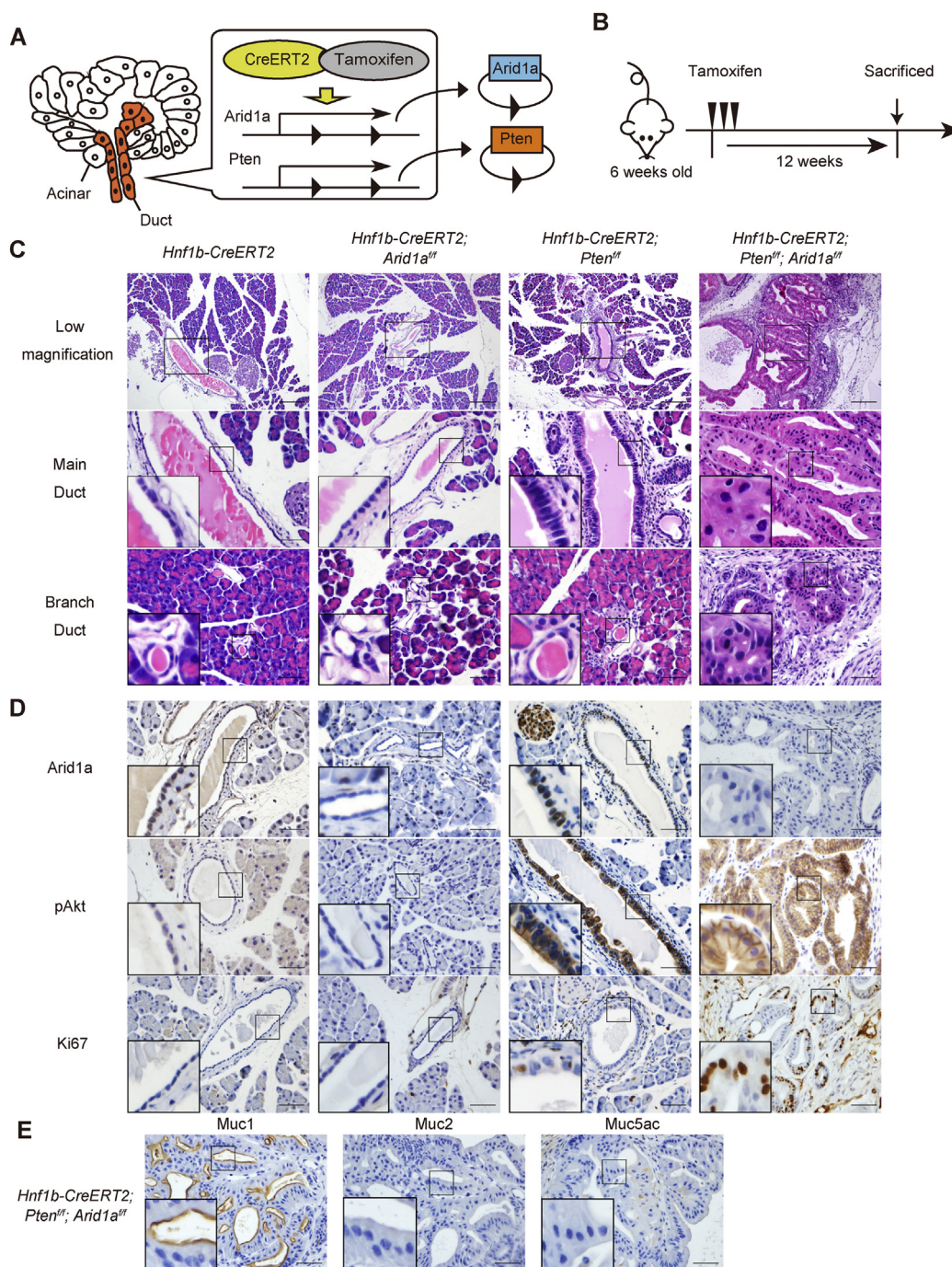


Figure 1. Pancreatic ductal cell-specific loss of *Arid1a* and *Pten* induces ITPN and ITPN-derived PDAC in mice. (A) Scheme illustrating pancreatic ductal cell-specific genetic recombination in HPA mice. (B) Experimental design of tamoxifen administration and analysis. Arrowheads indicate tamoxifen administration. The mice were killed 12 weeks after the first tamoxifen administration. (C) Representative image of hematoxylin and eosin (H&E) staining of the pancreas from *H*, *HA*, *HP*, and *HPA* mice. Top: Histologic images of the pancreas around the main pancreatic duct at low magnification; middle: high magnification image of the boxed regions in the top panels; bottom: histologic images of the pancreas around the branch duct at high magnification. Scale bars, 200 μ m (low magnification) and 50 μ m (high magnification). *H*, *HA*, and *HP*: *n* = 3, *HPA*: *n* = 5. (D) IHC for *Arid1a*, pAkt, and Ki67 in the pancreas of *H*, *HA*, *HP*, and *HPA* mice. Scale bar, 50 μ m. *H*, *HA*, and *HP*: *n* = 3, *HPA*: *n* = 5. (E) IHC for Muc1, Muc2, and Muc5ac in the ITPN lesions of *HPA* mice. Scale bar, 50 μ m. *n* = 5.

mice became moribund approximately 20 weeks after tamoxifen administration most likely due to hepatic dysfunction, as these mice showed massive hepatic tumors

and jaundice (data not shown). These data indicate that simultaneous deletion of *Pten* and *Arid1a* in pancreatic ductal cells results in the development of pancreatic ductal

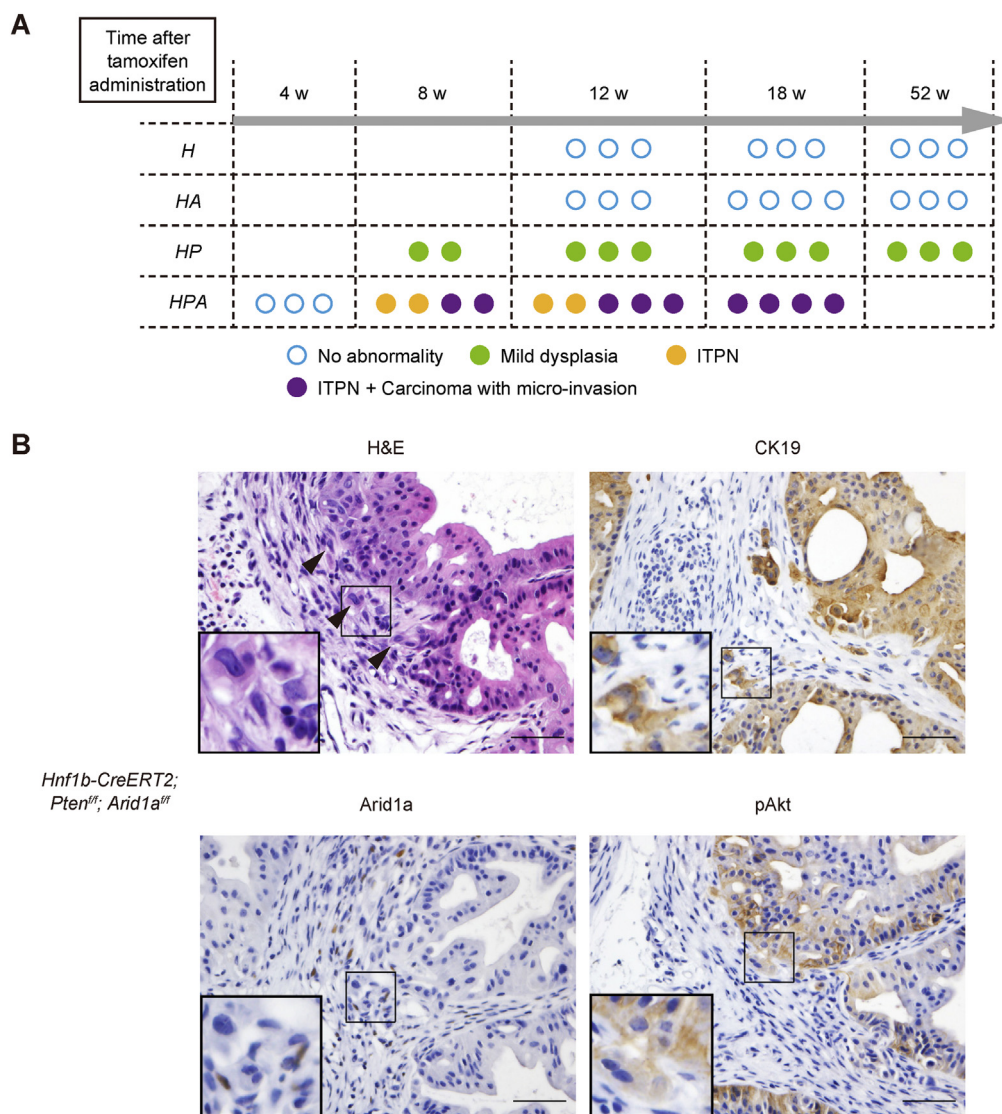


Figure 2. Simultaneous loss of *Arid1a* and *Pten* in pancreatic ductal cell leads to the development of PDAC with microinvasion. (A) Summary of the timeline and phenotypes in pancreas phenotypes after tamoxifen administration. Each circle represents an individual pancreatic sample. w, week. (B) Representative images of hematoxylin-eosin (H&E) staining and IHC for CK19, *Arid1a*, and pAkt in PDAC with microinvasion in *HPA* mice 12 or 18 weeks after tamoxifen administration. Arrowheads indicate microinvasion of PDAC cells. Scale bar, 50 μm.

tumors that closely resemble human ITPN and ITPN-derived PDAC.

Loss of *Arid1a* and *Pten* leads to Dedifferentiation of Pancreatic Ductal Cells

Cancer progression often involves dedifferentiation of epithelial cells, which is accompanied by an acquisition of progenitor and stem cell-like potentials.²⁶ Therefore, to investigate the molecular mechanism by which ITPNs form from pancreatic ductal cells in the concurrent absence of *Arid1a* and *Pten*, we evaluated the expression of mature ductal cell markers, *Sox9* and *Hnf1β*,^{23,27,28} and pancreatic precursor cell markers, *Pdx1*, *Aldh1a1*, and *Cd133* by immunostaining.^{29,30} Notably, the expression of mature ductal cell markers was impaired in ITPNs of *HPA* mice but observed in the ductal cells of *H*, *HA*, and *HP* mice. In contrast, expression levels of the pancreatic precursor cell markers were elevated in ITPN lesions of *HPA* mice compared with ductal cells in *H*, *HA*, and *HP* mice (Figure 3A

and Supplementary Figure 2A). These results suggest that simultaneous loss of *Arid1a* and *Pten* induces the dedifferentiation of ductal cells.

For further molecular analysis, we generated pancreatic ductal cell organoids from the pancreas of *H*, *HA*, *HP*, and *HPA* mice 4 weeks after tamoxifen administration. These pancreatic ductal cell organoids were roundish and did not show any apparent differences in morphology. Quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) showed that the expression of *Pten* in *HP* and *HPA* organoids and the expression of *Arid1a* in *HA* and *HPA* organoids was dramatically diminished (Supplementary Figure 2B). The expression levels of pancreatic precursor cell markers, including *Aldh1a1*, *Sox17*, *Hnf4a*, and *Cd133*, were elevated in organoids derived from *HPA* mice compared with those from other genotypes (Figure 3C and Supplementary Figure 2B). On the other hand, the expression levels of ductal cell marker genes, including *Sox9* and *Hnf1β*, were not different between all genotypes. We also found that the expression of embryonic stem cell marker genes, including

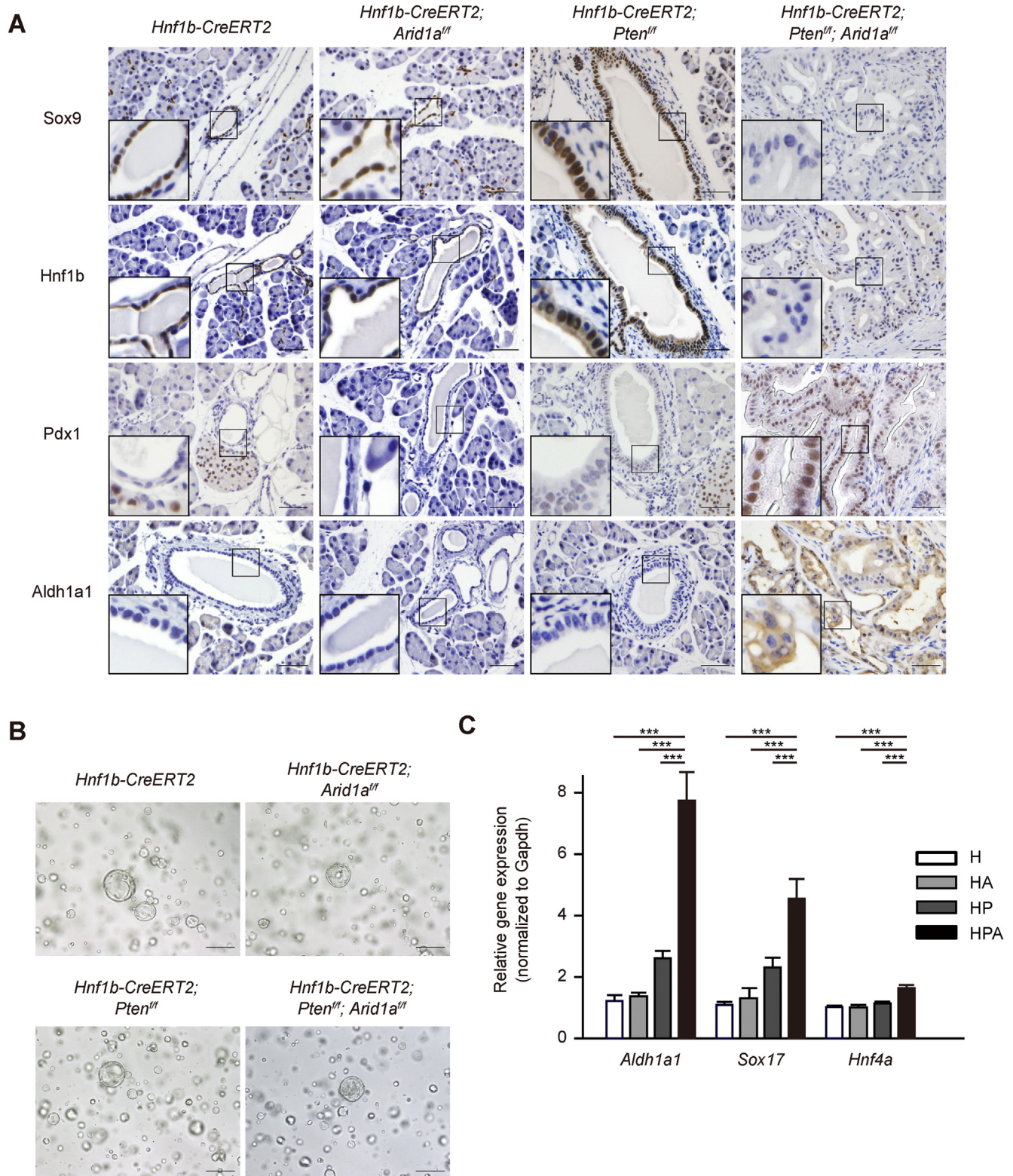


Figure 3. Dual loss of Arid1a and Pten induces dedifferentiation of pancreatic ductal cells. (A) IHC for Sox9, Hnf1b, Pdx1, and Aldh1a1 in the pancreas of *H*, *HA*, *HP*, and *HPA* mice 12 or 18 weeks after tamoxifen administration. Scale bar, 50 μ m. *H* and *HP*: $n = 6$, *HA*: $n = 7$, *HPA*: $n = 9$. (B) Images of organoid culture of pancreatic ductal cells isolated from *H*, *HA*, *HP*, and *HPA* mice 12 weeks after tamoxifen administration. Scale bar, 50 μ m. (C) qRT-PCR of pancreatic precursor cell marker genes in pancreatic ductal organoids. Data are presented as the mean $\pm$ SD ($n = 3$ per group). *** $P < .001$ (1-way analysis of variance with Tukey's test).

Sox2, *Nanog*, and *Oct3/4*, were almost undetectable, and the expression of *Klf4* was comparable among *H*, *HA*, *HP*, and *HPA* organoids (Supplementary Figure 2B). These data suggest that concurrent inactivation of *Arid1a* and *Pten* induces dedifferentiation of pancreatic ductal cells into a pancreatic precursor-like state, but not into a more primitive state such as an embryonic stem cell-like state in vitro and in vivo.

Characterization of Pancreatic Ductal Cells of *HPA* Mice by RNA-seq Analysis

Our data showed that *HPA* mice developed ITPN-like lesions and that *HP* mice did not develop premalignant lesions. These data indicate that aberrant activation of the PI3K/Akt pathway per se is not sufficient to induce ITPN formation and that concurrent loss of *Arid1a* is required for ITPN development. To identify the molecular features caused by loss of *Arid1a* in pancreatic ductal cells, we next performed RNA-seq of pancreatic ductal cells isolated from whole pancreas tissue using ductal cell-specific Dolichos biflorus agglutinin lectin labeling followed by magnetic bead separation (Figure 4A).³¹ RNA-seq analysis of genes with high fold changes ($> 2 \log$ or $< -2 \log$) revealed 734 upregulated and 258 downregulated genes in *HPA* mice (Figure 4B). Gene Set Enrichment Analysis (GSEA) using “Hallmark gene sets” showed that Myc target genes, E2F target genes, and EMT-related genes were enriched in ductal cells from *HPA* mice compared with ductal cells from *H* and *HP* mice, consistent with the findings of previous studies on *Arid1a* knockout mice (Figure 4C).^{20,32,33} We also found that genes involved in oxidative phosphorylation and fatty acid metabolism were enriched in ductal cells from *H* and *HP* mice, whereas those involved in glycolysis were elevated in ductal cells from *HPA* mice (Figure 4C and Supplementary Figure 3), suggesting that loss of *Arid1a* may induce metabolic reprogramming in pancreatic ductal cells, as previously reported in other organs.^{34,35} Results from GSEA using “Ontology gene sets” showed that several pathways associated with endoderm development were enriched in *HPA* mice (Figure 4D), suggesting that pancreatic ductal cells in *HPA* mice possess a dedifferentiating potential that fits the in vivo phenotypes observed for this mouse (Figures 1 and 3). We performed qRT-PCR analysis to confirm these changes in in vitro organoids. The expression of glycolysis-related genes, such as *Hk2* and *Slc16a3*, were increased in *HPA* organoids compared with *HP* organoids, whereas those of EMT-related genes and Myc target genes were comparable between *HP* and *HPA* organoids (Supplementary Figure 2).

Simultaneous Loss of *Arid1a* and *Pten* Induces YAP/TAZ-TEAD Pathway Activation in Pancreatic Ductal Cells

Aberrant activation of the Yes-associated protein 1/Transcriptional coactivator with PDZ-binding motif (YAP/TAZ)-TEAD pathway is observed in various types of cancers, including PDAC.^{36,37} Consistent with this, results from GSEA showed that YAP activity signatures were significantly

enriched in pancreatic ductal cells of *HPA* mice compared with *H* and *HP* mice (Figure 5A and Supplementary Figure 3). We confirmed that expression of *Ctgf*, *Ankrd1*, and *Birc5*—well-known target genes of the YAP/TAZ-TEAD complex—were elevated in *HPA* pancreatic ductal cells, as determined by qRT-PCR (Figure 5B) and IHC (Figure 5C) using in vitro organoids and in vivo pancreatic tissues, respectively. Of note, we found that the expression of these YAP/TAZ-TEAD target genes was not different between *H* and *HA* organoids, suggesting that single deletion of *Arid1a* did not alter the expression of YAP/TAZ target genes in pancreatic ductal cells. In addition, we found that nuclear staining of TAZ, but not YAP, was increased in ITPN lesions in *HPA* mice compared with pancreatic ductal cells in *HP* mice, which is consistent with the increased expression of YAP/TAZ-TEAD target genes in *HPA* mice (Figure 5C). Taken together, these data indicate that the YAP/TAZ-TEAD pathway is activated in the pancreatic ductal cells of *HPA* mice but not in those of *H*, *HA*, and *HP* mice.

ARID1A Is Essential for the Direct Binding of the SWI/SNF Complex and YAP/TAZ in Pancreatic Ductal Cells and Can Suppress YAP/TAZ-TEAD Activity

Given that *Arid1a* is a core subunit of the SWI/SNF complex that regulates gene expression through chromatin structure remodeling, we hypothesized that the elevated expression of YAP/TAZ-TEAD target genes and subsequent dedifferentiation in *HPA* mice resulted from changes in chromatin accessibility due to the loss of *Arid1a*. To test this, we performed an ATAC-seq of pancreatic ductal cell organoids and found that the chromatin status of YAP/TAZ-TEAD target genes did not differ between *HP* and *HPA* organoids (Figure 5E).

Western blot analysis of co-immunoprecipitation assays revealed that YAP/TAZ directly interacted with Brg1, a core subunit of the SWI/SNF complex, in pancreatic ductal cell organoids from *HP* mice (Figure 5F and G). By contrast, this interaction was impaired in pancreatic ductal cell organoids from *HPA* mice, suggesting that ARID1A is essential for the direct interaction of the SWI/SNF complex with YAP/TAZ protein. The findings indicate that loss of *Arid1a*-mediated interaction between the SWI/SNF complex and YAP/TAZ leads to dysregulated activation of the YAP/TAZ-TEAD pathway, subsequently promoting tumorigenesis through ITPN/PDAC development in *HPA* mice.

The YAP/TAZ Pathway in Mice Is Activated in ITPN But Not in IPMN

We reported that *Ptf1a-Cre*; *Kras*^{G12D}; *Arid1a*^{f/f} (PKA) mice developed IPMN, and we further showed that IPMN in these mice originated from pancreatic ductal cells. Given that both ITPN and IPMN arise from pancreatic ductal cells, we next investigated the molecular mechanism underlying ITPN development in *HPA* mice. In contrast to the aberrant activation of the YAP/TAZ-TEAD pathway in the pancreatic ductal cells of *HPA* mice—indicated by elevated expression

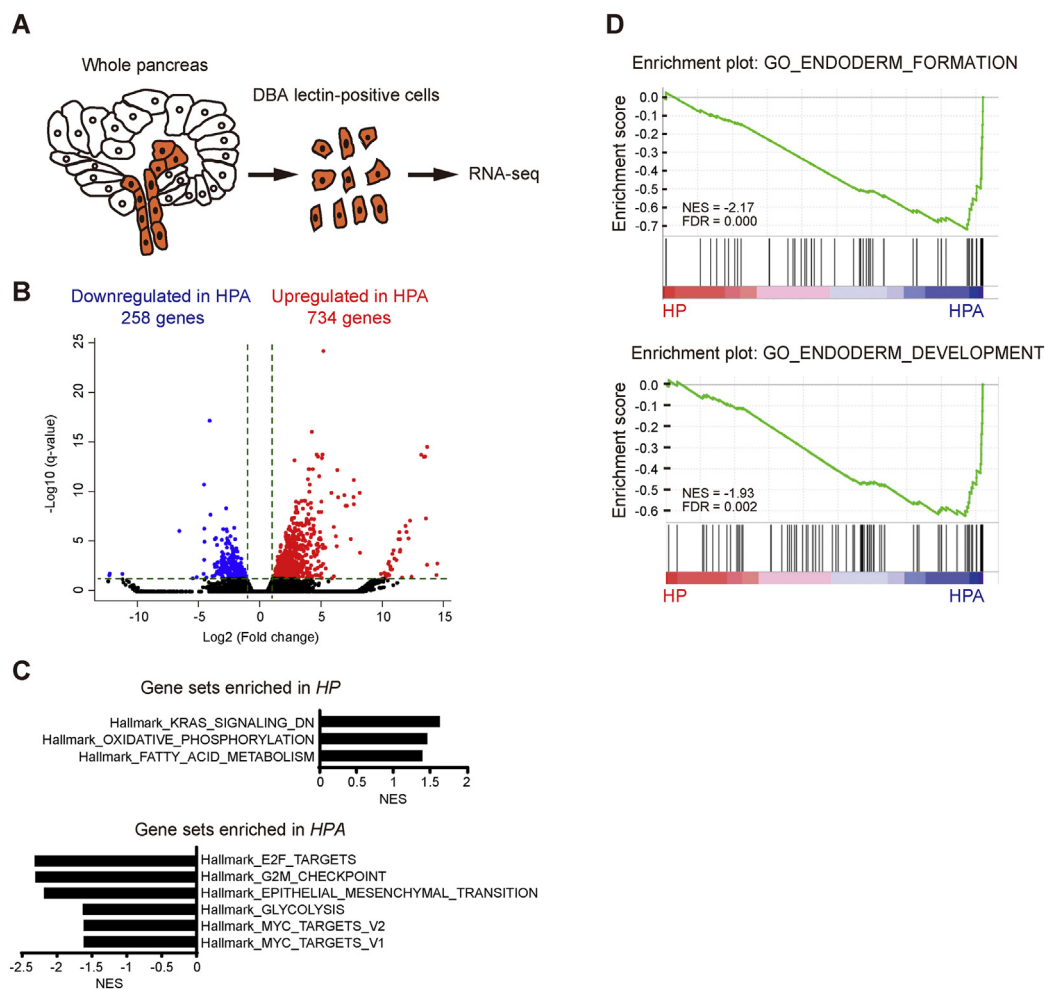


Figure 4. RNA-seq analysis using pancreatic ductal cells isolated from the mouse pancreas. (A) Schematic illustrating pancreatic ductal cell isolation from the murine whole pancreas using ductal cell-specific Dolichos biflorus agglutinin (DBA) lectin labeling. (B) Volcano plot showing the differentially expressed genes in organoids from each genotype. Each dot indicates individual genes. (C) GSEA of pancreatic ductal cells from *HP* and *HPA* mice using “Hallmark gene sets.” NES, normalized enrichment score. (D) GSEA enrichment plots. FDR, false discovery rate.

of target genes of the YAP/TAZ-TEAD pathway (*Ctgf* and *Birc5*) and increased expression of TAZ in ITPN—IPMN in *Ptf1a-Cre; Kras^{G12D}; Arid1a^{fl/fl}* (*PKA*) mice did not show increased expression of target genes of the YAP/TAZ-TEAD pathway and TAZ (Figure 6A). It is known that the PI3K/Akt pathway induces activation of YAP/TAZ-TEAD pathway.^{38,39} To investigate the potential influence of the PI3K/Akt pathway on the YAP/TAZ-TEAD activity, we performed IHC for pAkt in *HPA* and *PKA* mice. We found that the expression level of pAkt was elevated in *HPA* mice compared with *PKA* mice (Supplementary Figure 4). These data indicate that augmented protein expression and transcriptional activity of the YAP/TAZ-TEAD complex are distinct features of ITPN in *HPA* mice, and are not observed in IPMN of *PKA* mice.

TAZ Expression in Humans Is Elevated in ITPN and ITPN-derived PDAC But Not in IPMN

To establish the human relevance of ITPN tumors developed in *HPA* mice, we investigated YAP/TAZ pathway activation in human ITPN and ITPN-derived PDAC samples

as well as normal adjacent pancreas or IPMN samples by histology. In good agreement with our mouse data, we found that TAZ was highly expressed with nuclear localization in all tested ITPN and ITPN-derived PDAC samples but was absent in IPMN and normal adjacent samples (Figure 6B). Expression levels of YAP, pAkt, and ARID1A, were not different between ITPNs and IPMNs (Figure 6C and D and Supplementary Figure 5). Consistent with our mouse data, the data suggest that the activated TAZ-TEAD pathway can distinguish ITPNs and ITPN-derived PDACs from IPMNs in humans.

The YAP/TAZ Pathway Functionally Mediates the Dedifferentiation of Pancreatic Ductal Cells and ITPN Formation in *HPA* Mice

YAP/TAZ-TEAD regulates the enhancer network of human embryonic pancreatic progenitors.⁴⁰ We thus hypothesized that increased YAP/TAZ activity leads to the dedifferentiation of pancreatic ductal cells in *HPA* mice as the mechanism driving ITPN/PDAC tumorigenesis. To test

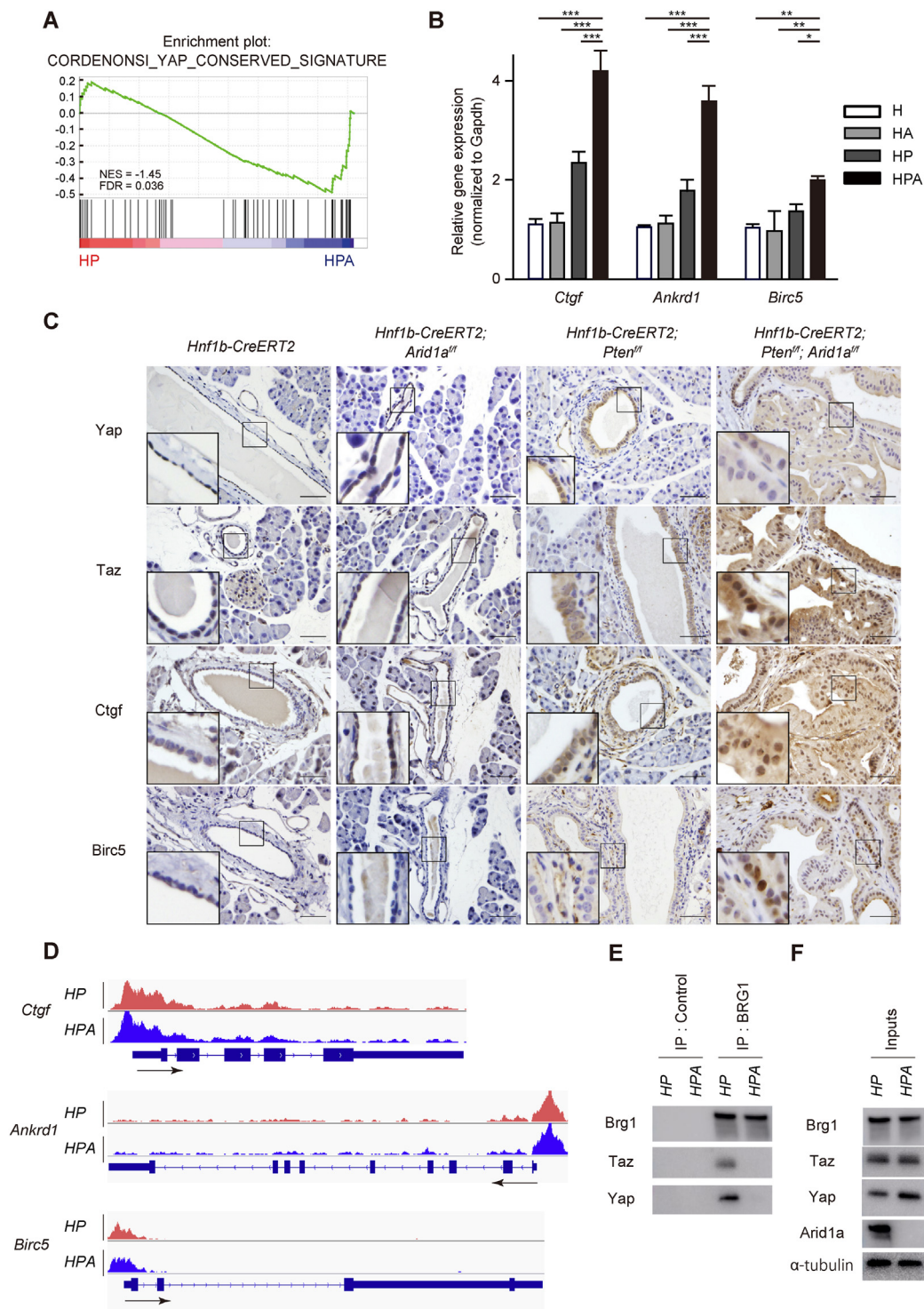


Figure 5. Dual loss of *Arid1a* and *Pten* activates the YAP/TAZ-TEAD pathway in pancreatic ductal cells. (A) GSEA enrichment plot. Normalized enrichment score (NES) and false discovery rate (FDR) q values are shown. (B) qRT-PCR of YAP/TAZ-TEAD complex target genes in pancreatic ductal organoids. Data are presented as the mean $\pm$ SD (n = 3 per group). * P < .05, ** P < .01, *** P < .001 (1-way analysis of variance with Tukey's test). (C) IHC for Yap, Taz, Ctgf, and Birc5 in the pancreas of *H*, *HA*, *HP*, and *HPA* mice 12 weeks after tamoxifen administration. Scale bar, 50 μ m. *H* and *HP*: n = 6, *HA*: n = 7, *HPA*: n = 9. (D) IGV view of ATAC-seq analysis at the locus of representative YAP/TAZ-TEAD target genes in *HP* and *HPA* organoids (n = 2). Arrows indicate the transcriptional orientation of each gene. (E) Western blotting of the co-immunoprecipitation experiment with *HP* and *HPA* organoids using anti-Brg1 or isotype control antibodies. Secondary antibodies are indicated on the left. (F) Western blotting of the inputs of the co-immunoprecipitation experiments in (E).

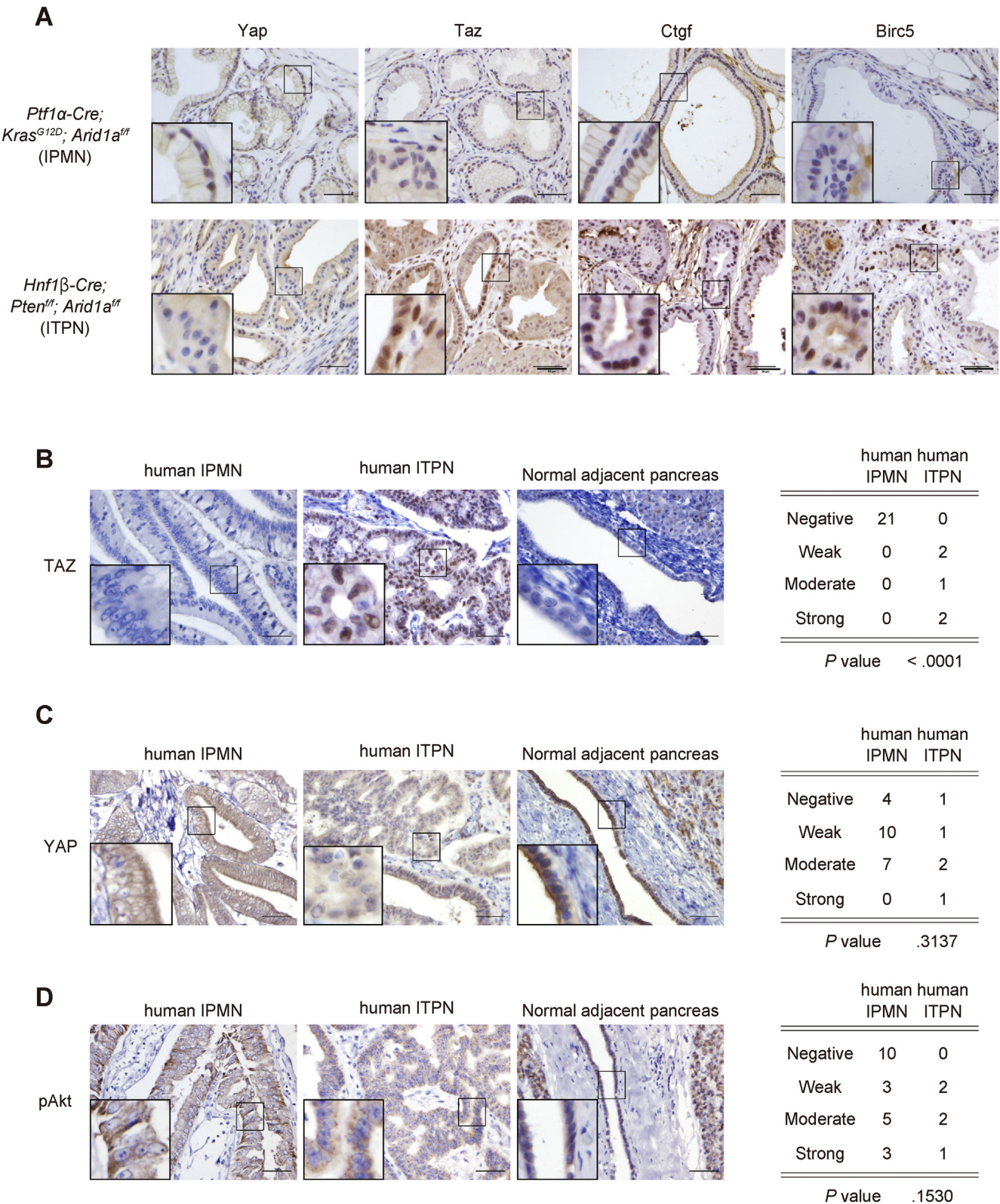


Figure 6. TAZ expression is activated in human ITPNs and ITPN-derived PDACs but not in IPMNs. (A–D) IHC for Yap, Taz, Ctgf, and Birc5 in the pancreas of *Ptf1α-Cre; Kras^{G12D}; Arid1a^{fl/fl}* mice 45 to 52 weeks after birth and *HPA* mice 12 weeks after tamoxifen administration. Scale bar, 50 μm. n = 3. (B–D) IHC for TAZ (B), YAP (C), and pAkt (D) in IPMN (n = 21), ITPN (n = 5), and adjacent normal pancreas (n = 3) specimens. Images of the adjacent normal pancreas were captured from the same tissue slide as ITPN if available. Scale bar, 50 μm. Tables on the right show the number of samples classified as ITPN or IPMN and their staining intensity. Statistical analysis was performed using the Mann-Whitney test.

this, we used verteporfin (VP), a chemical compound that inhibits the interaction between YAP/TAZ and TEAD.⁴¹ As expected, VP treatment of pancreatic ductal cell organoids reduced the expression of target genes of the YAP/TAZ-TEAD complex and glycolysis-related genes, as determined by qRT-PCR (Figure 7A and Supplementary Figure 2). Furthermore, VP treatment reduced the expression of pancreatic precursor cell markers, including *Sox17*, *Hnf4a*, and *Aldh1a1*, in a dose-dependent manner (Figure 7A).

To investigate the role of the YAP/TAZ-TEAD pathway in ITPN development in vivo, we administered either VP or vehicle to *HPA* mice intraperitoneally for 4 weeks (Figure 7B). Histopathological analysis revealed that all *HPA* mice in the vehicle-treated group developed ITPN and carcinoma in situ of the pancreas, and 1 of the 3 *HPA* mice developed PDAC with microinvasion. In contrast, neither ITPN, carcinoma in situ, nor PDAC with microinvasion was observed in VP-treated *HPA* mice (Figure 7C and D). Consistent with the data from organoid experiments, *Sox9* and *Pdx1* expression was increased and decreased, respectively, in pancreatic ductal cells in VP-treated mice compared with vehicle-treated mice, indicating that inhibition of the YAP/TAZ-TEAD pathway suppresses the dedifferentiation of pancreatic ductal cells in *HPA* mice (Figure 7E). Overall, the findings indicate that activation of the YAP/TAZ pathway functionally mediates the dedifferentiation of pancreatic ductal cells, further leading to the development of ITPN and ITPN-derived PDAC in *HPA* mice.

Discussion

In this study, we demonstrated for the first time that concurrent loss of *Arid1a* and *Pten* in pancreatic ductal cells in adult mice results in the formation of ITPN and ITPN-derived PDAC with microinvasion. We explored the molecular mechanisms by which the loss of these 2 genes contributes to ITPN development, and found that their simultaneous loss in pancreatic ductal cells leads to aberrant activation of the YAP/TAZ pathway, which induces dedifferentiation of pancreatic ductal cells into precursor-like cells; this is critical for the development of ITPN-derived PDAC. TAZ expression was also found elevated in human ITPNs and ITPN-derived PDACs, but not in IPMNs, indicating that upregulation of TAZ expression is a distinctive feature of ITPN. Furthermore, pharmacological inhibition of the YAP/TAZ pathway prevented the dedifferentiation of pancreatic ductal cells and development of ITPN and ITPN-derived PDAC. The pancreatic intraductal neoplasms found in *Arid1a* and *Pten* deleted mice closely resembled human ITPN in terms of histological features, mucin expression patterns, and the premalignant nature of progression into PDAC. Thus, we established for the first time a genetically engineered mouse model of ITPN and ITPN-derived PDAC, highlighting the importance of the synergistic effect of *Arid1a* loss and PI3K/AKT pathway activation in the specification of PDAC precursors and development of PDAC.

We found that loss of *Arid1a* in the context of activated PI3K/Akt pathway induced activation of the YAP/TAZ

pathway in pancreatic ductal cells. Previous studies revealed that the PI3K/Akt pathway positively regulates the YAP/TAZ pathway through multiple signaling pathways,^{38,42,43} and another study showed a positive correlation between the expression of *PIK3CA* and YAP target genes in patients with head and neck squamous cell carcinoma.⁴⁴ Moreover, a recent study showed that the SWI/SNF complex interacts with and inhibits the YAP/TAZ pathway by directly binding to YAP/TAZ through ARID1A⁴⁵; the authors proposed that 2 requirements must be met to fully activate the YAP/TAZ responses: promotion of YAP/TAZ localization in the nucleus and inhibition of ARID1A-SWI/SNF activity. In agreement with this, we found that a single deletion of *Arid1a* or *Pten* did not alter YAP/TAZ target gene expression in pancreatic ductal cells, whereas concurrent deletion of *Arid1a* and *Pten* significantly induced YAP/TAZ-TEAD target gene expression. We also demonstrated direct binding of the SWI/SNF complex to YAP and TAZ in pancreatic ductal cells. Furthermore, our ATAC-seq data showed that the chromatin structure of YAP/TAZ-TEAD target genes did not change in pancreatic ductal cell organoids from *HP* and *HPA* mice, suggesting that loss of *Arid1a* induces YAP/TAZ activation by suppressing the ARID1A-SWI/SNF-YAP/TAZ inhibitory axis and not through chromatin remodeling, which is a well-known function of the SWI/SNF complex. Taking all these findings into consideration, our data suggest that activation of the PI3K/Akt pathway and *Arid1a* deletion synergistically promote YAP/TAZ pathway activation.

We found that the expression of glycolysis-related genes was increased in pancreatic ductal cells from *HPA* mice compared with those from *HP* mice. The expression of these genes were suppressed by inhibition of the YAP/TAZ-TEAD pathway, suggesting that the elevated expression of glycolysis-related genes was caused by aberrant activation of the YAP/TAZ-TEAD pathway. Regarding the relationship between glycolysis and dedifferentiation or ITPN development, high rates of glycolysis even in the presence of oxygen, a phenomenon known as the Warburg effect, is prevalent in pluripotency and appears to be dynamically regulated to facilitate the differentiation process.⁴⁶ Future studies are required to investigate the contribution of changes in cellular metabolism to dedifferentiation of pancreatic ductal cells and ITPN development.

We also demonstrated that inhibition of the YAP/TAZ-TEAD pathway suppressed the dedifferentiation of pancreatic ductal cells and inhibited the development of ITPN and ITPN-derived PDAC in *HPA* mice. Consistent with this, a recent study demonstrated that aberrant activation of YAP in pancreatic ductal cells is sufficient to induce rapid transformation,⁴⁷ supporting the notion that the YAP/TAZ pathway plays an important role in the development of premalignant lesions of PDAC. However, a previous report showed that *Sox9* expression is sustained in pancreatic ductal cells after induction of aberrant YAP/TAZ activation and that this aberrant activation results in the formation of PanIN but not ITPN.⁴⁷ These data suggest that additional changes, including loss of *Arid1a*, may be required to induce dedifferentiation of pancreatic ductal cells and specification toward ITPN development. In addition, a previous study

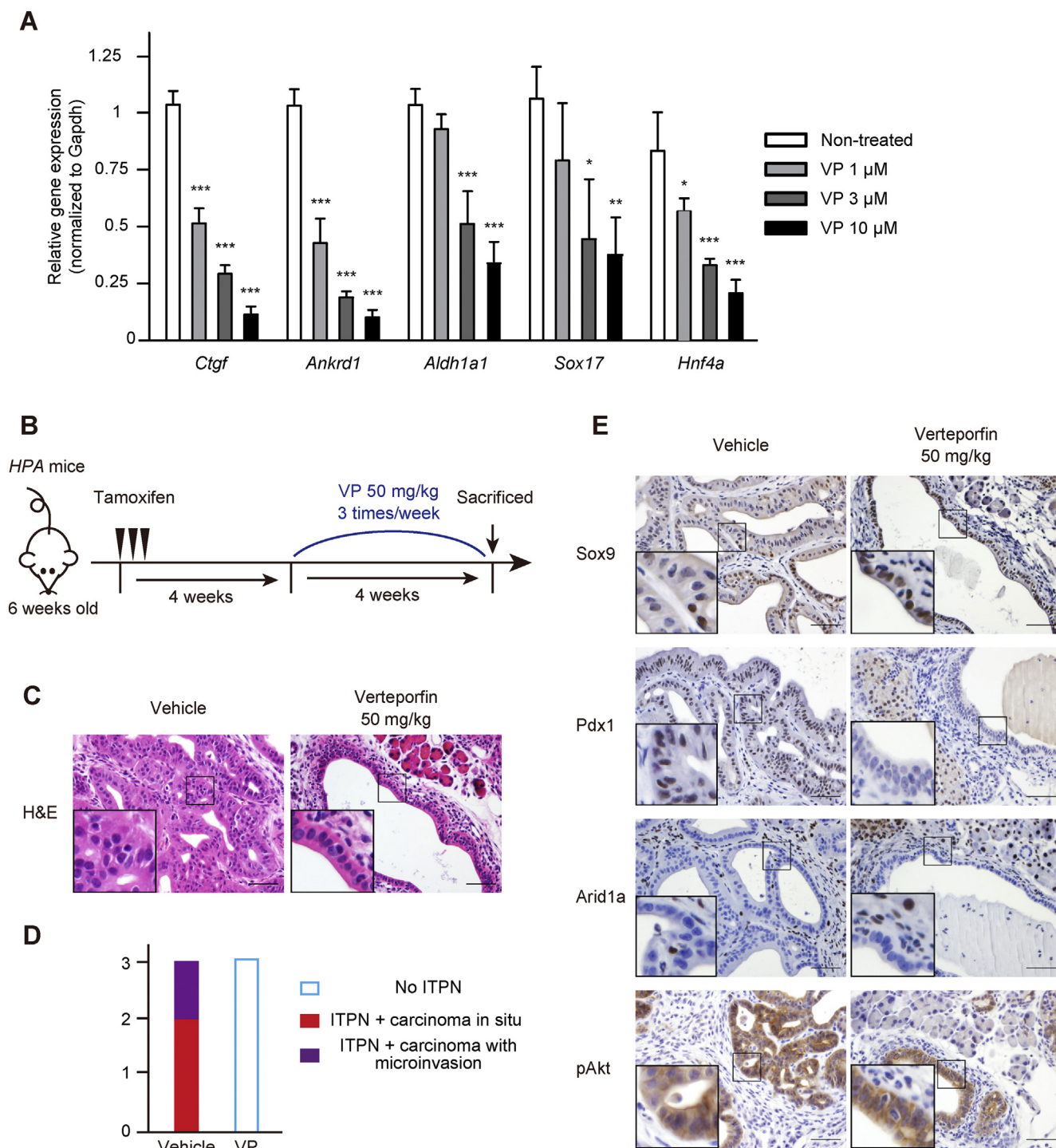


Figure 7. Inhibition of the YAP/TAZ-TEAD complex suppresses dedifferentiation of pancreatic ductal cells and ITPN formation in *HPA* mice. (A) qRT-PCR of YAP/TAZ-TEAD complex target genes and pancreatic precursor cell marker genes in pancreatic ductal organoids treated with dimethyl sulfoxide (control) or verteporfin (VP) for 48 hours. Data are presented as the mean $\pm$ SD ($n = 3$ per group). * $P < .05$, ** $P < .01$, *** $P < .001$ vs control (1 way analysis of variance with Dunnett's test). (B) Experimental design of the analysis and administration of tamoxifen and VP. Arrowheads indicate tamoxifen administration. (C) Representative hematoxylin-eosin (H&E) staining of the pancreas from vehicle- and VP-treated mice ($n = 3$ per group). (D) Quantification of histopathological analysis of pancreas from vehicle- and VP-treated mice ($n = 3$ per group). (E) IHC for Sox9, Pdx1, Arid1a, and pAkt in the pancreas of vehicle- and VP-treated mice. Scale bar, 50 μ m ($n = 3$ per group).

showed that *Ptf1a-Cre; Kras^{G12D}; Gnas^{R201C}* mice developed IPMN- and IPMN-derived PDAC, which was more differentiated and expressed more genes associated with ductal

phenotypes. They also reported that YAP1 was inactivated in cells from *Ptf1a-Cre; Kras^{G12D}; Gnas^{R201C}* mice through Hippo pathway activation. Given that Hippo pathway

negatively regulates the both YAP and TAZ, these data are consistent with our conclusion that aberrant activation of YAP/TAZ-TEAD induces dedifferentiation of pancreatic ductal cells and ITPN development.

A previous study reported that the activating mutation of the PI3K/Akt pathway in pancreatic ductal cells leads to the formation of IPMN but not ITPN in mice.⁴⁸ Potential reasons for this contradictory result is the difference in TAZ activity. In line with the previous report showing that the PI3K/Akt pathway positively regulates the YAP/TAZ pathway,^{38,42,43} we found that the expression level of pAkt was elevated in ITPN compared with IPMN in mice. These data suggest the potential contribution of the PI3K/Akt pathway in the ITPN development in *HPA* mice. On the other hand, in human tissues, the expression level of pAkt was not different between IPMN and ITPN. These data suggest that aberrant activation of the PI3K/Akt pathway is important, but not sufficient for specification toward ITPN. The role of PI3K/Akt pathway activation in the ITPN development needs to be investigated in future studies. We observed elevated TAZ expression in both mouse and human ITPNs but not in mouse and human IPMNs. In support of our findings, several genes that were upregulated in human ITPN, including *MLL1* and *BAP1*, were reported to be involved in YAP/TAZ pathway activation.^{49,50} Furthermore, *FGFR2*, whose fusion gene was found in 18% of ITPN cases,⁹ is important for YAP activation in cholangiocarcinoma.⁵¹ Our data, together with previous studies, highlight that activation of the YAP/TAZ pathway is a distinctive feature of ITPN and ITPN-derived PDAC.

Of note, we found that the expression level of ARID1A protein was not different between ITPN and IPMN in humans, whereas the expression of Arid1a was completely lost in *HPA* mice due to *Arid1a* deletion. The possible explanation for this inconsistency is that the YAP/TAZ pathway could be activated by various pathways other than loss of ARID1A. It is known that the YAP/TAZ pathway is regulated by variety of cellular signaling such as the Hippo pathway, Wnt pathway, and PI3K/Akt pathway. Therefore, it is likely that YAP/TAZ expression and activity is elevated through the mechanisms other than loss of ARID1A in human ITPNs. Interestingly, the expression of TAZ, but not YAP, was elevated in mouse and human ITPNs. Although they are generally considered functionally redundant, increasing evidence supports differential roles for YAP and TAZ in many contexts, from organ morphogenesis and tissue homeostasis to cancer development and progression.³⁹ YAP and TAZ activity is strictly regulated by several post-translational modifications that control their stability and intracellular localization. Recent studies have revealed that there are paralog-specific regulatory mechanisms to regulate the activity of YAP and TAZ. For example, S58 and S62 on TAZ, which are not present in YAP, can be phosphorylated by glycogen synthase kinase 3 (GSK3), creating a binding site for β -TrCP and leading to TAZ degradation through the ubiquitination pathway.³⁹ Further studies are needed to reveal the precise mechanisms underlying the elevated protein expression and functional importance of TAZ in the pathogenesis of ITPN.

In summary, the findings revealed the functional roles of Arid1a and the PI3K/Akt pathway in the development of PDAC, which arises via ITPN, a precursor PDAC lesion subtype. Specifically, loss of Arid1a and activation of the PI3K/Akt pathway in pancreatic ductal cells synergistically induce dedifferentiation of pancreatic ductal cells and the development of ITPN and ITPN-derived PDAC; ARID1A acts as a tumor suppressor in the context of an activated PI3K/Akt pathway through regulation of the YAP/TAZ pathway; and TAZ expression is elevated in human ITPN and ITPN-derived PDAC samples but not in mouse and human IPMNs. Thus, our findings provide novel insights into the molecular pathogenesis of ITPN and ITPN-derived PDAC and highlight the YAP/TAZ pathway as a potential therapeutic target.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at <https://doi.org/10.1053/j.gastro.2022.04.020>.

References

1. Hidalgo M, Cascinu S, Kleeff J, et al. Addressing the challenges of pancreatic cancer: future directions for improving outcomes. *Pancreatol* 2015;15:8–18.
2. Yamaguchi H, Shimizu M, Ban S, et al. Intraductal tubulopapillary neoplasms of the pancreas distinct from pancreatic intraepithelial neoplasia and intraductal papillary mucinous neoplasms. *Am J Surg Pathol* 2009; 33:1164–1172.
3. Kim JY, Hong SM. Precursor lesions of pancreatic cancer. *Oncol Res Treat* 2018;41:603–610.
4. Nagtegaal ID, Odze RD, Klimstra D, et al. The 2019 WHO classification of tumours of the digestive system. *Histopathology* 2020;76:182–188.
5. Kanda M, Matthaei H, Wu J, et al. Presence of somatic mutations in most early-stage pancreatic intraepithelial neoplasia. *Gastroenterology* 2012;142:730–733.e9.
6. Cancer Genome Atlas Research Network. Electronic address aadhe, Cancer Genome Atlas Research N. Integrated Genomic Characterization of Pancreatic Ductal Adenocarcinoma. *Cancer Cell* 2017;32:185–203.e13.
7. Springer S, Wang Y, Dal Molin M, et al. A combination of molecular markers and clinical features improve the classification of pancreatic cysts. *Gastroenterology* 2015;149:1501–1510.
8. Chang XY, Wu Y, Li Y, et al. Intraductal papillary mucinous neoplasms of the pancreas: clinical association with KRAS. *Mol Med Rep* 2018;17:8061–8068.
9. Basturk O, Berger MF, Yamaguchi H, et al. Pancreatic intraductal tubulopapillary neoplasm is genetically distinct from intraductal papillary mucinous neoplasm and ductal adenocarcinoma. *Mod Pathol* 2017; 30:1760–1772.
10. Shain AH, Pollack JR. The spectrum of SWI/SNF mutations, ubiquitous in human cancers. *PLoS One* 2013;8: e55119.

11. Kadoch C, Hargreaves DC, Hodges C, et al. Proteomic and bioinformatic analysis of mammalian SWI/SNF complexes identifies extensive roles in human malignancy. *Nat Genet* 2013;45:592–601.
12. Witkiewicz AK, McMillan EA, Balaji U, et al. Whole-exome sequencing of pancreatic cancer defines genetic diversity and therapeutic targets. *Nat Commun* 2015;6:6744.
13. Wilson BG, Roberts CW. SWI/SNF nucleosome remodelers and cancer. *Nat Rev Cancer* 2011;11:481–492.
14. Sausen M, Phallen J, Adleff V, et al. Clinical implications of genomic alterations in the tumour and circulation of pancreatic cancer patients. *Nat Commun* 2015;6:7686.
15. Zhang L, Wang C, Yu S, et al. Loss of ARID1A expression correlates with tumor differentiation and tumor progression stage in pancreatic ductal adenocarcinoma. *Technol Cancer Res Treat* 2018;17:1533034618754475.
16. Zhang X, Zhang Y, Yang Y, et al. Frequent low expression of chromatin remodeling gene ARID1A in breast cancer and its clinical significance. *Cancer Epidemiol* 2012;36:288–293.
17. Zhu YP, Sheng LL, Wu J, et al. Loss of ARID1A expression is associated with poor prognosis in patients with gastric cancer. *Hum Pathol* 2018;78:28–35.
18. Wu JN, Roberts CW. ARID1A mutations in cancer: another epigenetic tumor suppressor? *Cancer Discov* 2013;3:35–43.
19. Kimura Y, Fukuda A, Ogawa S, et al. ARID1A maintains differentiation of pancreatic ductal cells and inhibits development of pancreatic ductal adenocarcinoma in mice. *Gastroenterology* 2018;155:194–209.e2.
20. Wang W, Friedland SC, Guo B, et al. 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. *Gut* 2019;68:1245–1258.
21. Chandler RL, Damrauer JS, Raab JR, et al. Coexistent ARID1A-PIK3CA mutations promote ovarian clear-cell tumorigenesis through pro-tumorigenic inflammatory cytokine signalling. *Nat Commun* 2015;6:6118.
22. Suryo Rahmanto Y, Shen W, Shi X, et al. Inactivation of Arid1a in the endometrium is associated with endometrioid tumorigenesis through transcriptional reprogramming. *Nat Commun* 2020;11:2717.
23. Solar M, Cardalda C, Houbracken I, et al. Pancreatic exocrine duct cells give rise to insulin-producing beta cells during embryogenesis but not after birth. *Dev Cell* 2009;17:849–860.
24. Gao X, Tate P, Hu P, et al. ES cell pluripotency and germ-layer formation require the SWI/SNF chromatin remodeling component BAF250a. *Proc Natl Acad Sci U S A* 2008;105:6656–6661.
25. Adsay NV. Intraductal neoplasms of the pancreas. In: Bosman FT, et al, eds. WHO classification of tumours of the digestive system. World Health Organization, 2010:304–313.
26. Malta TM, Sokolov A, Gentles AJ, et al. Machine learning identifies stemness features associated with oncogenic dedifferentiation. *Cell* 2018;173:338–354.e15.
27. Kopp JL, Dubois CL, Schaffer AE, et al. Sox9⁺ ductal cells are multipotent progenitors throughout development but do not produce new endocrine cells in the normal or injured adult pancreas. *Development* 2011;138:653–665.
28. Seymour PA. Sox9: a master regulator of the pancreatic program. *Rev Diabet Stud* 2014;11:51–83.
29. Bastidas-Ponce A, Scheibner K, Lickert H, et al. Cellular and molecular mechanisms coordinating pancreas development. *Development* 2017;144:2873–2888.
30. Socorro M, Criscimanna A, Riva P, et al. Identification of newly committed pancreatic cells in the adult mouse pancreas. *Sci Rep* 2017;7:17539.
31. Reichert M, Takano S, Heeg S, et al. Isolation, culture and genetic manipulation of mouse pancreatic ductal cells. *Nat Protoc* 2013;8:1354–1365.
32. Sun X, Chuang JC, Kanchwala M, et al. Suppression of the SWI/SNF component Arid1a promotes mammalian regeneration. *Cell Stem Cell* 2016;18:456–466.
33. Wang SC, Nassour I, Xiao S, et al. SWI/SNF component ARID1A restrains pancreatic neoplasia formation. *Gut* 2019;68:1259–1270.
34. Ogiwara H, Takahashi K, Sasaki M, et al. Targeting the vulnerability of glutathione metabolism in ARID1A-deficient cancers. *Cancer Cell* 2019;35:177–190.e8.
35. Lissanu Deribe Y, Sun Y, Terranova C, et al. Mutations in the SWI/SNF complex induce a targetable dependence on oxidative phosphorylation in lung cancer. *Nat Med* 2018;24:1047–1057.
36. Zanonato F, Cordenonsi M, Piccolo S. YAP/TAZ at the roots of cancer. *Cancer Cell* 2016;29:783–803.
37. Rozengurt E, Sinnett-Smith J, Eibl G. Yes-associated protein (YAP) in pancreatic cancer: at the epicenter of a targetable signaling network associated with patient survival. *Signal Transduct Target Ther* 2018;3:11.
38. Zhao Y, Montminy T, Azad T, et al. PI3K positively regulates YAP and TAZ in mammary tumorigenesis through multiple signaling pathways. *Mol Cancer Res* 2018;16:1046–1058.
39. Reggiani F, Gobbi G, Ciarrocchi A, et al. YAP and TAZ are not identical twins. *Trends Biochem Sci* 2021;46:154–168.
40. Cebola I, Rodriguez-Segui SA, Cho CH, et al. TEAD and YAP regulate the enhancer network of human embryonic pancreatic progenitors. *Nat Cell Biol* 2015;17:615–626.
41. Moroishi T, Hansen CG, Guan KL. The emerging roles of YAP and TAZ in cancer. *Nat Rev Cancer* 2015;15:73–79.
42. Fan R, Kim NG, Gumbiner BM. Regulation of Hippo pathway by mitogenic growth factors via phosphoinositide 3-kinase and phosphoinositide-dependent kinase-1. *Proc Natl Acad Sci U S A* 2013;110:2569–2574.
43. Kim NG, Gumbiner BM. Adhesion to fibronectin regulates Hippo signaling via the FAK-Src-PI3K pathway. *J Cell Biol* 2015;210:503–515.
44. Garcia-Escudero R, Segrelles C, Duenas M, et al. Overexpression of PIK3CA in head and neck squamous cell carcinoma is associated with poor outcome and activation of the YAP pathway. *Oral Oncol* 2018;79:55–63.

45. Chang L, Azzolin L, Di Biagio D, et al. The SWI/SNF complex is a mechanoregulated inhibitor of YAP and TAZ. *Nature* 2018;563:265–269.
 46. Reid MA, Dai Z, Locasale JW. The impact of cellular metabolism on chromatin dynamics and epigenetics. *Nat Cell Biol* 2017;19:1298–1306.
 47. Park J, Eisenbarth D, Choi W, et al. YAP and AP-1 cooperate to initiate pancreatic cancer development from ductal cells in mice. *Cancer Res* 2020;80:4768–4779.
 48. Kopp JL, Dubois CL, Schaeffer DF, et al. Loss of Pten and activation of Kras synergistically induce formation of intraductal papillary mucinous neoplasia from pancreatic ductal cells in mice. *Gastroenterology* 2018;154:1509–1523.e5.
 49. Zhang H, Khoa LTP, Mao F, et al. MLL1 inhibition and vitamin D signaling cooperate to facilitate the expanded pluripotency state. *Cell Rep* 2019;29:2659–2671.e6.
 50. Lee HJ, Pham T, Chang MT, et al. The tumor suppressor BAP1 regulates the Hippo pathway in pancreatic ductal adenocarcinoma. *Cancer Res* 2020;80:1656–1668.
 51. Rizvi S, Yamada D, Hirsova P, et al. A Hippo and fibroblast growth factor receptor autocrine pathway in cholangiocarcinoma. *J Biol Chem* 2016;291:8031–8047.
- Akihisa Fukuda, MD, PhD (Conceptualization: Supporting; Writing – review & editing: Supporting).
 Mayuki Omatsu, MD (conducted the experiments and analyzed the data: Supporting).
 Mio Namikawa, MD (conducted the experiments and analyzed the data: Supporting).
 Makoto Sono, MD (conducted the experiments and analyzed the data: Supporting).
 Tomonori Masuda, MD (conducted the experiments and analyzed the data: Supporting).
 Osamu Araki, MD (conducted the experiments and analyzed the data: Supporting).
 Munemasa Nagao, MD (conducted the experiments and analyzed the data: Supporting).
 Takaaki Yoshikawa, MD (conducted the experiments and analyzed the data: Supporting).
 Satoshi Ogawa, PhD (conducted the experiments and analyzed the data: Supporting).
 Yukiko Hiramatsu, PhD (conducted the experiments and analyzed the data: Supporting).
 Yu Muta, PhD (conducted the experiments and analyzed the data: Supporting).
 Motoyuki Tsuda, PhD (conducted the experiments and analyzed the data: Supporting).
 Takahisa Maruno, PhD (conducted the experiments and analyzed the data: Supporting).
 Yuki Nakanishi, PhD (conducted the experiments and analyzed the data: Supporting).
 Jorge Ferrer, PhD (Resources: Supporting).
 Tatsuki Tsuruyama, PhD (diagnosed murine neoplasia of the pancreas: Supporting).
 Toshihiko Masui, PhD (Resources: Supporting).
 Etsuro Hatano, PhD (Resources: Supporting).
 Hiroshi Seno, PhD (Writing – review & editing: Supporting).

Conflicts of interest

This author discloses the following: Yuichi Fukunaga is employed by Sumitomo Dainippon Pharma Co., Ltd. The remaining authors disclose no conflicts.

Funding

This work was supported in part by Grants-in-Aid KAKENHI (19H03639, 19K16712, 19K22619, 20H03659). It was also supported by the Japan Agency for Medical Research and Development, the Project for Cancer Research and Therapeutic Evolution (18cm0106142h0001, 20cm0106177h0001, 20cm0106375h0001) and AMED-PRIME (20gm6010022h0003), Moonshot Research and Development Program (JPMJMS2022-1), and COI-NEXT (JPMJPF2018). It was also supported by Princess Takamatsu Cancer Research Fund (17-24924), the Myocarditis Foundation (2017bvAg), the Mitsubishi Foundation (201910037), the Uehara Foundation (201720143), the Naito Foundation (22205-1), the Kobayashi Foundation, the Shimizu Foundation, the Japan Foundation for Applied Enzymology, the Astellas Foundation, the SGH Foundation, the Kanae Foundation, Bristol Myers Squibb, the Ichiro Kanehara Foundation (20K1037), and the Takeda Foundation (201749741).

Received October 14, 2021. Accepted April 21, 2022.

Correspondence

Address correspondence to Akihisa Fukuda, MD, PhD, Department of Gastroenterology and Hepatology, Kyoto University Graduate School of Medicine, 54 Shogoin-Kawahara-cho, Sakyo-ku, Kyoto, 606-8507, Japan. e-mail: fukuda26@kuhp.kyoto-u.ac.jp.

Acknowledgments

The authors thank all members of the Fukuda laboratory for technical assistance and helpful discussions. We thank S. Narumiya for supervising Yuichi Fukunaga.

CRedit Authorship Contributions

Order of Authors (with Contributor Roles):

Yuichi Fukunaga, Master of Pharmacy (Conceptualization: Lead; Writing – original draft: Lead).

Supplementary Methods

Organoid Culture

Pancreatic ductal organoid cultures were established from the murine pancreas as described previously with slight modifications.¹ The mouse pancreas was minced into small pieces and digested with 1.5 mg/mL collagenase type V (Cat. no. C9263; Sigma-Aldrich) and 10 mg/mL DNase type 1 (Cat. no. DN25; Sigma-Aldrich) in Ad-DMEM/F12 (Cat. no. 12634-028; Invitrogen, Carlsbad, CA) at 37 °C for 15 to 20 minutes; the reaction was stopped by adding 0.9 g/L glucose and 47.6 μ M CaCl₂ in chilled Hank's Buffered Salt Solution (HBSS) (Cat. no. 14175-095; Invitrogen). Cells were centrifuged and the pellet was further digested with 0.05% trypsin-EDTA solution (Cat. no. 25300-054; Invitrogen) at room temperature for 5 minutes, and the reaction was stopped by adding chilled HBSS. The tissue suspension was passed through a 100- μ m cell strainer and washed twice with Ad-DMEM. Washed cells were mixed with 50 μ L Matrigel (Cat. no. 356231; Corning Inc., Corning, NY) per well in a 24-well plate and cultured with organoid medium. Passaging was performed at 1:4 to 1:8 split ratios twice a week. For passaging, organoids were digested in TrypLE (Invitrogen) for 10 minutes at 37 °C. Cells were centrifuged and the pellet was mixed with Matrigel and plated to a 24-well culture dish. The organoid medium consisted of Ad-DMEM/F12 supplemented with B27 (Cat. no. 17504-044; Thermo Fisher Scientific, Waltham, MA) and N2 (Cat. no. 17502-048; Thermo Fisher Scientific), 1X Glutamax (Cat. no. 35050-061; Thermo Fisher Scientific), 10 mM HEPES (Cat. no. 15630-080; Thermo Fisher Scientific), penicillin-streptomycin (Cat. no. 15070-063; Thermo Fisher Scientific), 1 mM N-acetylcysteine (Cat. no. A7250; Sigma-Aldrich), 10 mM nicotinamide (Cat. no. N0636; Sigma-Aldrich), 50 ng/mL epidermal growth factor (Cat. no. AF-100-15; Peprotech, Rosemont, IL), 1 μ g/mL Rspodin-1 (Cat. no. 120-38; Peprotech), 50 ng/mL Wnt3a (Cat. no. 5036-WN; R&D systems, Minneapolis, MN), 100 ng/mL Noggin (Cat. no. 121-10C; Peprotech), 100 ng/mL FGF10 (Cat. no. 100-26; Peprotech), 10 μ M Y-27632 (Cat. no. 1254; Tocris Bioscience, Bristol, UK), and 2 μ M SB431542 (Cat. no. 1614; Tocris Bioscience).

RNA isolation and qRT-PCR

Total RNA was extracted using the RNeasy Micro Kit (Qiagen, Hilden, Germany) and reverse transcribed to single-strand complementary DNA using the ReverTra Ace qRT-PCR RT Kit (TOYOBO, Tokyo, Japan). qRT-PCR was performed using the SYBR Green Master Mix (Roche Diagnostics, Mannheim, Germany) on a LightCycler 96 (Roche Diagnostics). Relative expression levels were determined by normalizing to Gapdh levels. Primer sequences are listed in [Supplementary Table 3](#).

RNA-seq and Analysis

The pancreases from 3 *HP* and 3 *HPA* mice were harvested 4 weeks after tamoxifen administration. Each

pancreas was digested as described in the organoid culture section. The filtrated cell suspension was incubated for 10 minutes on ice with 200 μ L of 1:200 diluted DBA lectin-fluorescein isothiocyanate (Cat. no. FL-1031; Vector Laboratories, Burlingame, CA) in sorting buffer (HBSS containing 0.5% bovine serum albumin [Sigma-Aldrich] and 2 mM EDTA [Invitrogen]). The cells were washed with 700 μ L sorting buffer, centrifuged, and then the pellet was incubated for 15 minutes on ice with anti-fluorescein isothiocyanate MicroBeads (Cat. no. 130-048-701; Miltenyi Biotec, Bergisch Gladbach, Germany). The cells were again washed in 1 mL sorting buffer and centrifuged, after which the pellet was resuspended in sorting buffer and magnetically sorted using an mass spectroscopy column (Cat. no. 130-042-201; Miltenyi Biotec). Isolated pancreatic ductal cells were used for RNA-seq, which was performed by Macrogen, Inc (Seoul, South Korea) on the Novaseq6000 platform with 2 $\times$ 100 base pair paired-end sequencing using SMART-Seq v4 Ultra Low Input RNA kit and TruSeq RNA Sample Prep Kit v2. Adaptors and low-quality bases were trimmed from the reads using Trimmomatic (version 0.39)² with default parameters. Reads were mapped to the *Mus musculus* reference genome build mm10 using STAR (version 2.7.3a)³ and counted by RSEM (version v1.3.1).⁴ Read count data were normalized using the iDEGES/edgeR method.⁵ Normalized count data were used for GSEA⁶ and differentially expressed genes were determined using TCC (Tag Count Comparison)⁵ with a false discovery rate cutoff value < 0.05.

ATAC-seq and Analysis

Pancreatic ductal cell organoids were harvested using TrypLE and washed twice with fresh medium, after which 1:100 volumes of 100X DNase buffer (250 mM MgCl₂ and 50 mM CaCl in H₂O) and 1:100 volumes of 100X DNase I solution (resuspended in HBSS at 20,000 units per mL; cat. no. LS002006; Worthington Biochemical Corporation, Lakewood, NJ) was added to the medium. Cells were incubated in DNase at 37°C for 30 minutes in a tissue culture incubator. After adding phosphate-buffered saline to the medium, cells were centrifuged and resuspended in 500 μ L of 50% fetal bovine serum/40% culture media/10% dimethyl sulfoxide (DMSO) solution in a slow cooling chamber. Approximately 50,000 cells were then shipped to Active Motif (Carlsbad, CA) for ATAC-seq and bioinformatic analysis.

Co-immunoprecipitation and Western Blotting

Co-immunoprecipitation was performed as previously described, with slight modifications.⁷ Cells were collected in lysis buffer (50 mM HEPES [pH 7.5], 100 mM NaCl, 50 mM KCl, 1% Triton X-100, 5% glycerol, 0.5% NP-40, 2 mM MgCl₂, 1 μ M DTT, and phosphatase and protease inhibitors) and lysed by sonication at 4 °C. The extracts were cleared by centrifugation, incubated with anti-BRG1 antibody (ab110641; Abcam, Cambridge, UK) or control rabbit immunoglobulin G (ab172730; Abcam), and immobilized on Dynabeads using the Dynabeads Antibody Coupling Kit (cat.

no. 14311D; Thermo Fisher Scientific) overnight at 4°C. The immunocomplexes were then collected using a magnet, washed with cold lysis buffer 3 times, resuspended in 2X Laemmli Sample Buffer (Bio-Rad Laboratories, Hercules, CA), incubated at 95°C for 5 minutes, and subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis and Western blot analysis. Proteins were run on 4% to 15% Mini-PROTEAN TGX gels (Bio-Rad Laboratories) and transferred onto polyvinyl difluoride membranes using the Trans-Blot Turbo Transfer System (Bio-Rad Laboratories). Membranes were incubated with blocking buffer (Blocking One; cat. no. 03953-95; Nacalai Tesque, Kyoto, Japan) for 1 hour at room temperature followed by the indicated primary antibodies at 4°C overnight. The samples were washed in 0.05% TBST buffer and probed with confirmation-specific horseradish peroxidase–conjugated secondary antibodies (1:2,000; #5124; Cell Signaling Technology, Danvers, MA) for 1 hour. Immunoblot signals were developed using the Super Signal West Pico PLUS Chemiluminescent Substrate (cat. no. 34577; Thermo Fisher Scientific) and imaged using the Amersham Imager 600 (GE Healthcare, Chicago, IL).

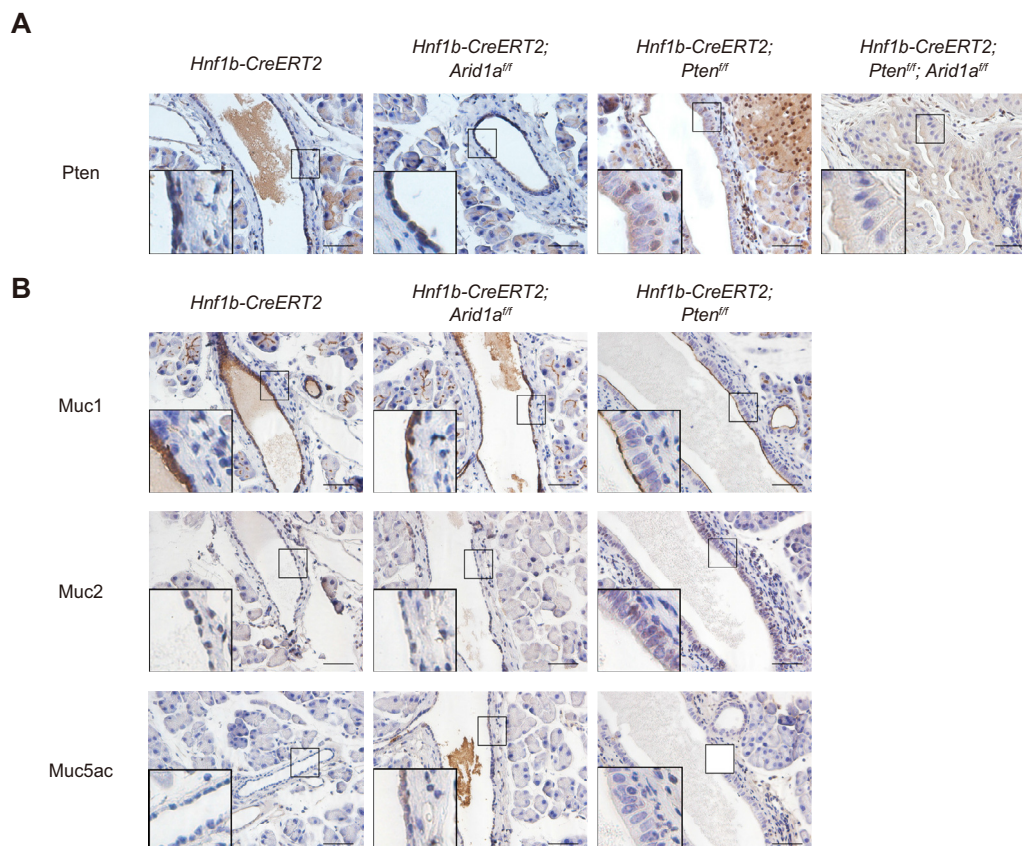
In Vivo VP Administration

VP (AOBIOUS, Gloucester, MA) was dissolved in corn oil containing 10% DMSO at a concentration of 5 mg/mL. Four weeks after tamoxifen administration, vehicle or VP was

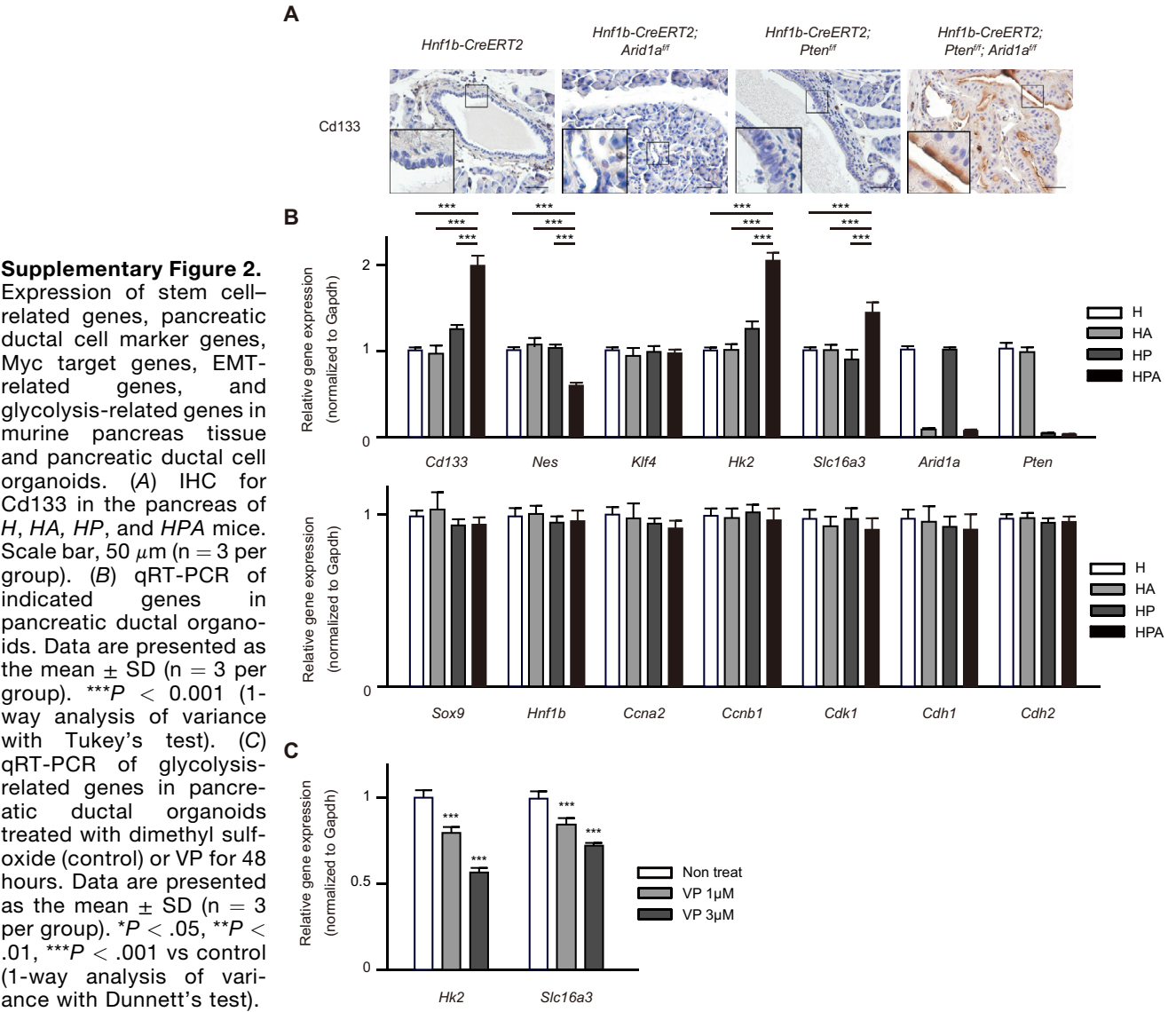
intraperitoneally injected 3 times a week at a dose of 50 mg/kg. After 4 weeks of treatment, the mice were killed, and pancreatic tissue was collected for histologic analysis.

Supplementary References

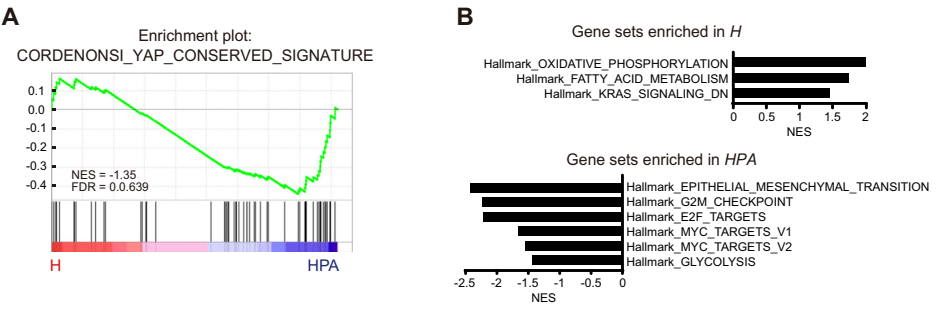
1. Reichert M, Takano S, Heeg S, et al. Isolation, culture and genetic manipulation of mouse pancreatic ductal cells. *Nat Protoc* 2013;8:1354–1365.
2. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 2014;30:2114–2120.
3. Dobin A, Davis CA, Schlesinger F, et al. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 2013;29:15–21.
4. Li B, Dewey CN. STAR: ultrafast universal RNA-seq aligner. *BMC Bioinformatics* 2011;12:323.
5. Sun J, Nishiyama T, Shumizu K, et al. TCC: an R package for comparing tag count data with robust normalization strategies. *BMC Bioinformatics* 2013;14:219.
6. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A* 2005;102:15545–15550.
7. Chang L, Azzolin L, Di Biagio D, et al. The SWI/SNF complex is a mechano-regulated inhibitor of YAP and TAZ. *Nature* 2018;563:265–269.

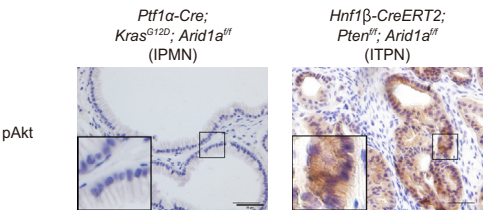


Supplementary Figure 1. Expression of Pten, Muc1, Muc2, and Muc5ac in mouse pancreas. (A) IHC for Pten in the pancreas of *H*, *HA*, *HP*, and *HPA* mice. Scale bar, 50 μ m ($n = 3$ per group). (B) IHC for Muc1, Muc2, and Muc5ac in the pancreas of *H*, *HA*, and *HP* mice. Scale bar, 50 μ m ($n = 3$ per group).

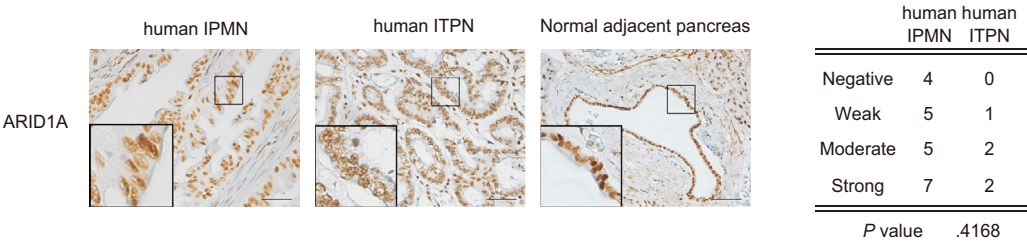


Supplementary Figure 3. GSEA between pancreatic ductal cells from *H* and *HPA* mice. (A) GSEA enrichment plot. NES and FDR q values are shown. (B) GSEA of pancreatic ductal cells from *HA* and *HPA* mice using “Hallmark gene sets.” FDR, false discovery rate; NES, normalized enrichment score.





Supplementary Figure 4. Expression of pAkt in mouse pancreas premalignant lesions. IHC for pAkt in the pancreas of *Ptf1α-Cre; Kras^{G12D}; Arid1a^{fl/fl}* mice 45 to 52 weeks after birth and *HPA* mice 12 weeks after tamoxifen administration. Scale bar, 50 μm, n = 3.



Supplementary Figure 5. Expression of ARID1A in human pancreas premalignant lesions. IHC for ARID1A in IPMN (n = 21), ITPN (n = 5), and adjacent normal pancreas (n = 3) specimens. Images of the adjacent normal pancreas were captured from the same tissue slide as ITPN if available. Scale bar, 50 μm. Tables on the right show the number of samples classified as ITPN or IPMN and their staining intensity. Statistical analysis was performed using the Mann-Whitney test.

Supplementary Table 1. Primary Antibodies for Immunohistochemistry

Antibodies	Source	Dilution	Catalog no.
ALDH1A1	Abcam	1:300	ab23375
CK19	Abcam	1:200	ab52625
HNF1 β	Proteintech	1:200	12533-1-AP
Ki67	BD Biosciences	1:400	550609
MUC1	Abcam	1:200	ab15481
MUC 2	SantaCruz	1:200	sc-15334
MUC5AC	Abcam	1:200	ab3649
ARID1A	Abcam	1:400	ab182560
phospho-Akt S475	Cell Signaling	1:200	#4060
SOX9	Millipore	1:1000	AB5535
PDX1	Abcam	1:10000	ab47308
YAP	CST	1:100	#14074
TAZ	CST	1:400	#72804
CTGF	Santa Cruz	1:100	sc-365970
Birc5	CST	1:400	#2808
PTEN	CST	1:125	#9188
CD133	Thermo Fisher	1:200	14-1331-82

Supplementary Table 2. Primary Antibodies for Western Blotting

Antibodies	Source	Dilution	Catalog no.
ARID1A	CST	1:1000	#12354
YAP	CST	1:1000	#14074
TAZ	CST	1:1000	#72804
BRG1	CST	1:1000	ab110641
α -tubulin	CST	1:1000	#2125

Supplementary Table 3.Primer Sets for Quantitative Reverse-Transcription Polymerase Chain Reaction Analyses

Gene symbol	Forward	Reverse
Gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
Aldh1a1	ATACTTGTGCGATTTAGGAGGCT	GGGCCTATCTTCCAAATGAACA
Ankrd1	GCTTAGAAGGACACTTGCGGATC	GACATCTGCGTTTCCTCCACGA
Birc5	GAGGCTGGCTTCATCCACTG	CTTTTGTCTGTTGTTGGTCTCC
Ctgf	TGCGAAGCTGACCTGGAGGAAA	CCGCAGAACTTAGCCCTGTATG
Hnf4a	TGCGAACTCCTTCTGGATGACC	CAGCACGTCTTAAACACCATGG
Sox17	GATGCGGGATACGCCAGTG	CCACCTCGCCTTTCACCTTTA
Cd133	CTGCGATAGCATCAGACCAAGC	CTTTTGACGAGGCTCTCCAGATC
Nes	AGGAGAAGCAGGGTCTACAGAG	AGTTCTCAGCCTCCAGCAGAGT
Klf4	CTATGCAGGCTGTGGCAAAACC	TTGCGGTAGTGCCTGGTCAGTT
Hk2	CCCTGTGAAGATGTTGCCCACT	CCTTCGCTTGCCATTACGCACG
Slc16a3	TCCATCCTGCTGGCTATGCTCT	CAGAAGGACGCAGCCACCATTG
Arid1a	TGTTGCCATGCATGTTGCTG	TGCCAGCATACTATTGATCCC
Pten	TGAGTTCCCTCAGCCATTGCCT	GAGGTTTCCTCTGGTCCTGGTA
Sox9	CACACGTCAAGCGACCCATGAA	TCTTCTCGCTCTCGTTCAGCAG
Hnf1b	GCCTTAGTGGAGGAGTGTAAACAG	TCTGCCTGAACGCCTCTTCCTT
Ccna2	TTGTAGGCACGGCTGCTATGCT	GGTGCTCCATTCTCAGAACCTG
Ccnb1	AGAGGTGGAACCTTGCTGAGCCT	GCACATCCAGATGTTCCATCGG
Cdk1	CATGGACCTCAAGAAGTACCTGG	CAAGTCTCTGTGAAGAACTCGCC
Cdh1	GGTCATCAGTGTGCTCACCTCT	GCTGTTGTGCTCAAGCCTTCAC
Cdh2	CCTCCAGAGTTTACTGCCATGAC	CCACCACTGATTCTGTATGCCG