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Tumor and Stem Cell Biology

Cancer Research

# Sirtuin-1 Regulates Acinar-to-Ductal Metaplasia and Supports Cancer Cell Viability in Pancreatic Cancer

Elke Wauters[2], Victor J. Sanchez-Arévalo Lobo[4], Andreia V. Pinho[1,4], Amanda Mawson[1], Daniel Herranz[5], Jianmin Wu[1], Mark J. Cowley[1], Emily K. Colvin[1], Erna Ngwayi Njicop[2], Rob L. Sutherland[1], Tao Liu[6], Manuel Serrano[5], Luc Bouwens[2], Francisco X. Real[4,7], Andrew V. Biankin[1], and Ilse Rooman[1,2,3]

## Abstract

The exocrine pancreas can undergo acinar-to-ductal metaplasia (ADM), as in the case of pancreatitis where precursor lesions of pancreatic ductal adenocarcinoma (PDAC) can arise. The $NAD^{+}$-dependent protein deacetylase Sirtuin-1 (Sirt1) has been implicated in carcinogenesis with dual roles depending on its subcellular localization. In this study, we examined the expression and the role of Sirt1 in different stages of pancreatic carcinogenesis, i.e. ADM models and established PDAC. In addition, we analyzed the expression of KIAA1967, a key mediator of Sirt1 function, along with potential Sirt1 downstream targets. Sirt1 was co-expressed with KIAA1967 in the nuclei of normal pancreatic acinar cells. In ADM, Sirt1 underwent a transient nuclear-to-cytoplasmic shuttling. Experiments where during ADM, we enforced repression of Sirt1 shuttling, inhibition of Sirt1 activity or modulation of its expression, all underscore that the temporary decrease of nuclear and increase of cytoplasmic Sirt1 stimulate ADM. Our results further underscore that important transcriptional regulators of acinar differentiation, that is, Pancreatic transcription factor-1a and β-catenin can be deacetylated by Sirt1. Inhibition of Sirt1 is effective in suppression of ADM and in reducing cell viability in established PDAC tumors. KIAA1967 expression is differentially downregulated in PDAC and impacts on the sensitivity of PDAC cells to the Sirt1/2 inhibitor Tenovin-6. In PDAC, acetylation of β-catenin is not affected, unlike p53, a well-characterized Sirt1-regulated protein in tumor cells. Our results reveal that Sirt1 is an important regulator and potential therapeutic target in pancreatic carcinogenesis. *Cancer Res; 73(7); 2357–67. ©2012 AACR.*

## Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer death in the United States, an outcome that has not changed for 50 years (1). Understanding the molecular mechanisms of PDAC initiation and tumor maintenance is imperative to develop chemoprevention and therapeutic strategies. New insights in PDAC initiation show that adult exocrine acinar cells under stress can dedifferentiate and gain metaplastic ductal characteristics (referred to as acinar-to-ductal metaplasia, ADM). There is compelling evidence from mouse models that ADM is a precursor lesion of PDAC (2, 3). ADM also occurs in pancreatitis, which may explain why pancreatitis is a major risk factor for PDAC (2, 3). Prevention of ADM and maintenance of acinar cell differentiation could suppress pancreatic carcinogenesis. Previously, we observed that nicotinamide repressed acinar cell dedifferentiation and ADM in culture (4). Nicotinamide is an end product and feedback inhibitor of Sirtuin-mediated protein deacetylation (5).

The repertoire of Sirtuin functions is broader than the role in longevity for which they were originally identified (6) and Sirtuins target a range of nuclear, mitochondrial, and cytoplasmic proteins. Sirtuins have multifaceted roles in cell death, differentiation, metabolism, and senescence. Sirtuin-1 (Sirt1), the best studied of the family, also plays roles in cancer and has been reported to be an oncogene as well as a tumor suppressor (7). Related to pancreas, Sirt1 has been studied in islets and diabetes (8, 9) (10), but only limited evidence exists that Sirt1 is important in PDAC (11).

One of the best-characterized regulators of Sirt1 is deleted in breast cancer 1 (Dbc1, KIAA1967; ref. 12). Dbc1 directly interacts with Sirt1 and inhibits Sirt1 activity (12, 13). Changes in either Dbc1 or Sirt1 expression result in altered Sirt1-driven effects.

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Because there was preliminary evidence that a Sirtuin inhibitor impairs ADM and Sirt1 is currently seen as a promising target of therapeutic intervention in other cancers (14), we aimed to study the expression of Sirt1 and Dbc1 in normal exocrine pancreas and during ADM to define the context-specific target genes of Sirt1 and to reveal Sirt1 effects in PDAC.

## Materials and Methods

### Cell cultures

Primary acinar cell culture protocols were adapted from previous works (15, 16). The cell lines 266-6 and AR42J-B13, the PDAC cell lines, and HEK293 cells were obtained from American Type Culture Collection (ATCC) Cell Biology Collection and used within 6 months between resuscitation and experimentation. The ATCC authentication protocols include testing for mycoplasma, bacteria, fungi contamination, confirmation of species identity, and detection of cellular contamination or misidentification using COI for interspecies identification and DNA profiling as well as cytogenetic analysis, flow cytometry, and immunocytochemistry with consistent refinement of cell growth conditions as well as documentation systems, ensuring traceability. Cells were cultured with leptomycin B (LMB; 0.25μg/mL, Sigma-Aldrich), nicotinamide (20–40 mmol/L, Sigma), resveratrol (50 mmol/L, Sigma), and Tenovin-6 (Cayman Chemical).

### Animals and *in vivo* experimentation

Pancreatitis and pancreatic duct ligation were conducted as described previously (17, 18) and approved by the ethics committee of the Vrije Universiteit Brussel (Brussels, Belgium) and the Garvan Animal Ethical Committee and were conducted in accordance with the Declaration of Helsinki as revised in 2000.

### Statistics

Results are presented as mean $\pm$ SEM. Data were analyzed by Prism 5.0 (Student *t* test or one-sample *t* test). Unless stated otherwise, the experiments were carried out at least 3 times independently. *P* values are indicated.

## Results

### Sirt1 and Dbc1 expression in normal exocrine tissue and ADM

We assessed the expression of Sirt1 and Dbc1 in normal pancreas and mouse models of ADM. Staining of Sirt1 in cells with the digestive enzyme amylase or with insulin illustrates that Sirt1 is expressed in the exocrine and endocrine pancreas, with only the latter reported previously (Fig. 1A; refs. 8, 9). Sirt1 and Dbc1 were co-expressed in the nuclei of exocrine pancreas (Fig. 1A). Expression was undetectable in about 30% of acinar cells (Supplementary Fig S1A).

In caerulein-induced acute pancreatitis (Cae-AP), acini undergo ADM and regenerate within 1 week (18). The ADM in our *in vivo* models corresponds to what has been described by Strobel and colleagues (19), that is, tubular complexes and mucinous metaplastic lesions (MML) and occurs isolated in the absence of pancreatic intraepithelial neoplasias (PanIN; ref. 20). The transient ADM is characterized by cytoplasmic retention of acinar enzymes, such as carboxypeptidase A1 (CpA1; Fig. 1B), increased expression and cytoplasmic accumulation of β-catenin and induction of ductal markers as keratin 19 (Krt19) (Supplementary Fig. S1B and S1C; refs. 18, 21). Nuclear Dbc1 staining did not change (Fig. 1B). In contrast, the Sirt1 staining observed in acini of untreated (Fig. 1A) and control PBS-treated pancreata (Fig. 1B) shifted from the nucleus to the cytoplasm within the first 2 days of Cae-AP (Fig. 1B). Nuclear Sirt1 expression was reestablished by day 8 (Fig. 1B). We noted that one third of the mice with Cae-AP showed longer lasting ADM with more cytoplasmic β-catenin and $Krt19^{+}$ complexes and more prominent cytoplasmic Sirt1 (Supplementary Fig. S1B).

Pancreatic duct ligation (PDL) is another model of injury with acinar tissue replaced by $Krt19^{+}$ complexes resulting from permanent ADM and ductal proliferation (ref. 17; Fig. 1C, Supplementary Fig. S2). Dbc1 expression remained unchanged (Supplementary Fig. S2B), but a nuclear-to-cytoplasmic shift of Sirt1 was again found (Fig. 1C). This shift was early and transient with restoration of nuclear expression within 1 week. The percentage of mice in which we observed cytoplasmic Sirt1 matched the success rate of PDL.

In conclusion, in the 2 *in vivo* models of ADM, the Dbc1 expression pattern was unchanged but an obvious intracellular shift of Sirt1 was noted, confined to the exocrine cells, that is, islet cells retained nuclear Sirt1 and Dbc1 (not shown). This observation suggests that in ADM, Sirt1 can have altered activity and effects on target proteins as a result of changed interaction with Dbc1 and changed intracellular localization.

### Functional significance of nuclear-to-cytoplasmic shuttling of Sirt1 in ADM

We reported before on rodent and human exocrine cell cultures (4, 15, 16), the latter specifically providing insights into onset of ADM that cannot be obtained from *in vivo* (clinical) samples. Cells of non-acinar origin that also can contribute to the *in vivo* ADM-type lesions are likely not represented in the *in vitro* models.

Again, we observed that, in contrast to the overall nuclear localization in normal tissue, the exocrine cells showed manifest cytoplasmic Sirt1 staining at the time of cell isolation (Fig. 2A for human and B for mouse). In human cultures, this was slightly more variable due to the variability in method and time of collection of donor tissue and the more elaborate isolation procedure, which allowed less control on the time point of analysis. Similar to the *in vivo* observations, nuclear Sirt1 expression became re-established during culture (Fig. 2A and B and Supplementary Fig. S3B) and nuclear localization of Dbc1 was unchanged (not shown).

We used the mouse ADM cultures for further functional analyses. No changes occur in levels of expression of Sirt1 or Dbc1 protein (Supplementary Fig. S3C). Within 24 hours, acinar cells dedifferentiate and undergo ADM comparable to the changes of Cae-AP in a panel of relevant genes



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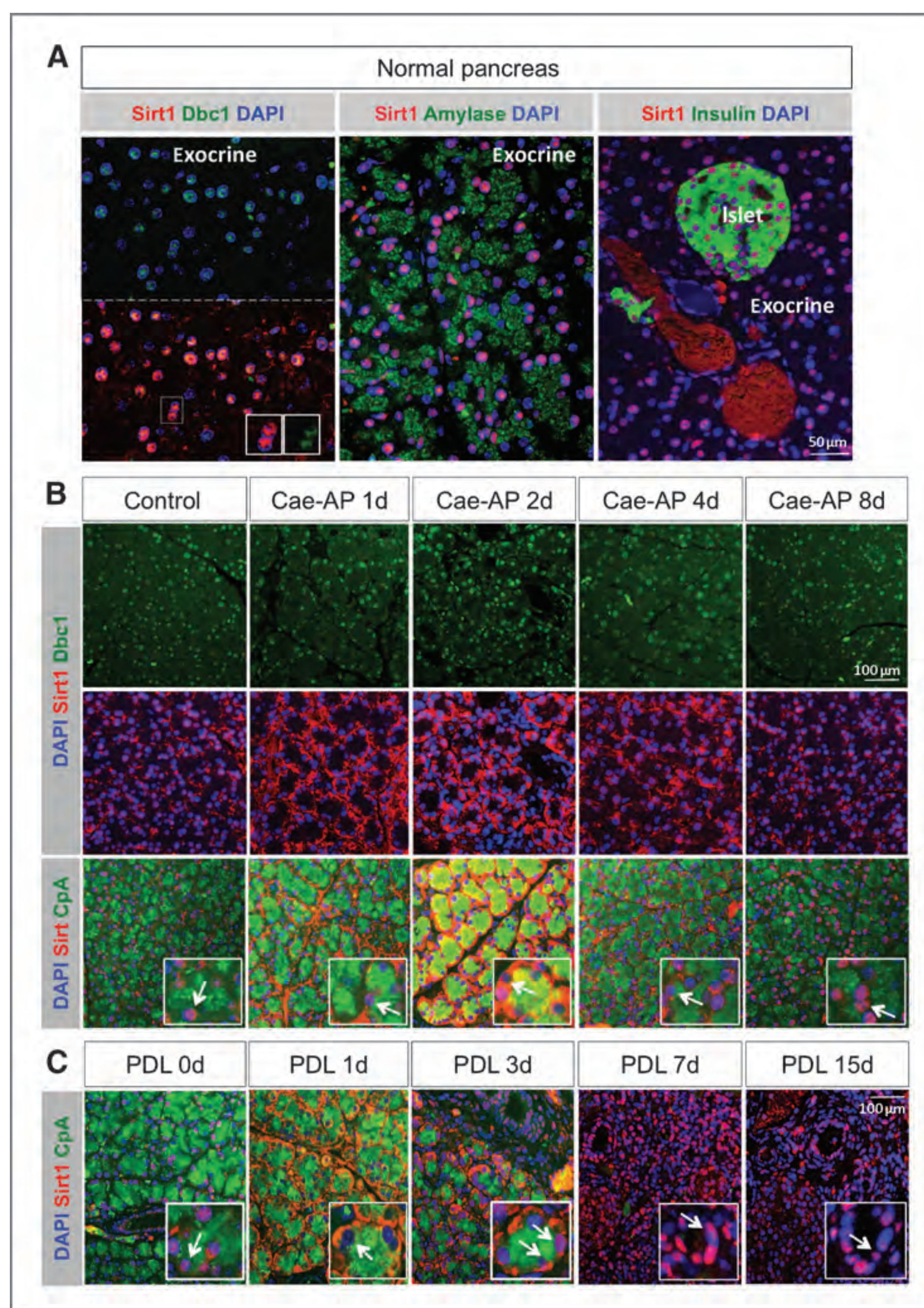


Figure 1. Sirt1 and Dbc1 expression in normal exocrine tissue and ADM. A, IF detection of Sirt1 and Dbc1 in normal mouse pancreas; co-localization of Dbc1 with Sirt1 (left), Sirt1 with the acinar cell marker amylase (middle), and Sirt1 with the endocrine hormone insulin (right). B, Sirt1 with Dbc1 or the acinar marker carboxypeptidase A (CpA) in caerulein-induced acute pancreatitis (Cae-AP 0–8 days). C, IF detection of Sirt1 and CpA in pancreatic duct ligation (PDL, 0–15 days). Pictures in a sequence are taken with the same exposure times. Inset, higher magnification and arrow points to the nuclear-to-cytoplasmic shuttling.

(Supplementary Fig. S3A), induction of Krt19 and changed β-catenin expression (Supplementary Fig. S3A and S3B).

To address the impact of nuclear-to-cytoplasmic shuttling of Sirt1, we treated the acinar cells with LMB, an antibiotic that inhibits nuclear export of proteins with a nuclear export signal, including Sirt1 (22). This resulted in more cells presenting nuclear Sirt1 staining (co-localization with Mist1 was used to label the acinar cell nuclei; Fig. 2C). Western blotting confirmed higher nuclear Sirt1 expression with LMB (Supplementary Fig. S3D). LMB restrained the induction of Krt19, a ductal marker that is strongly induced under control conditions, as seen in immunofluorescence (IF; Fig. 2C). Consistent with this, a lesser induction of *Krt19* mRNA was observed in LMB conditions (4.1 ± 0.8-fold vs. 10.9 ± 2.2-fold in controls, $P < 0.04$; Fig. 2D). No profound effect on the expression of acinar enzymes or transcription factors was found (not shown).

We explored further whether changes in Sirt1 expression impacted on acinar cell differentiation, starting with non-stress conditions. Pancreata from Pdx1-Cre;Sirt1$^{ex4lox/lox}$ mice show a mutant Sirt1 protein band that migrates slightly faster in Western blot analysis (Supplementary Fig. S4A), in line with



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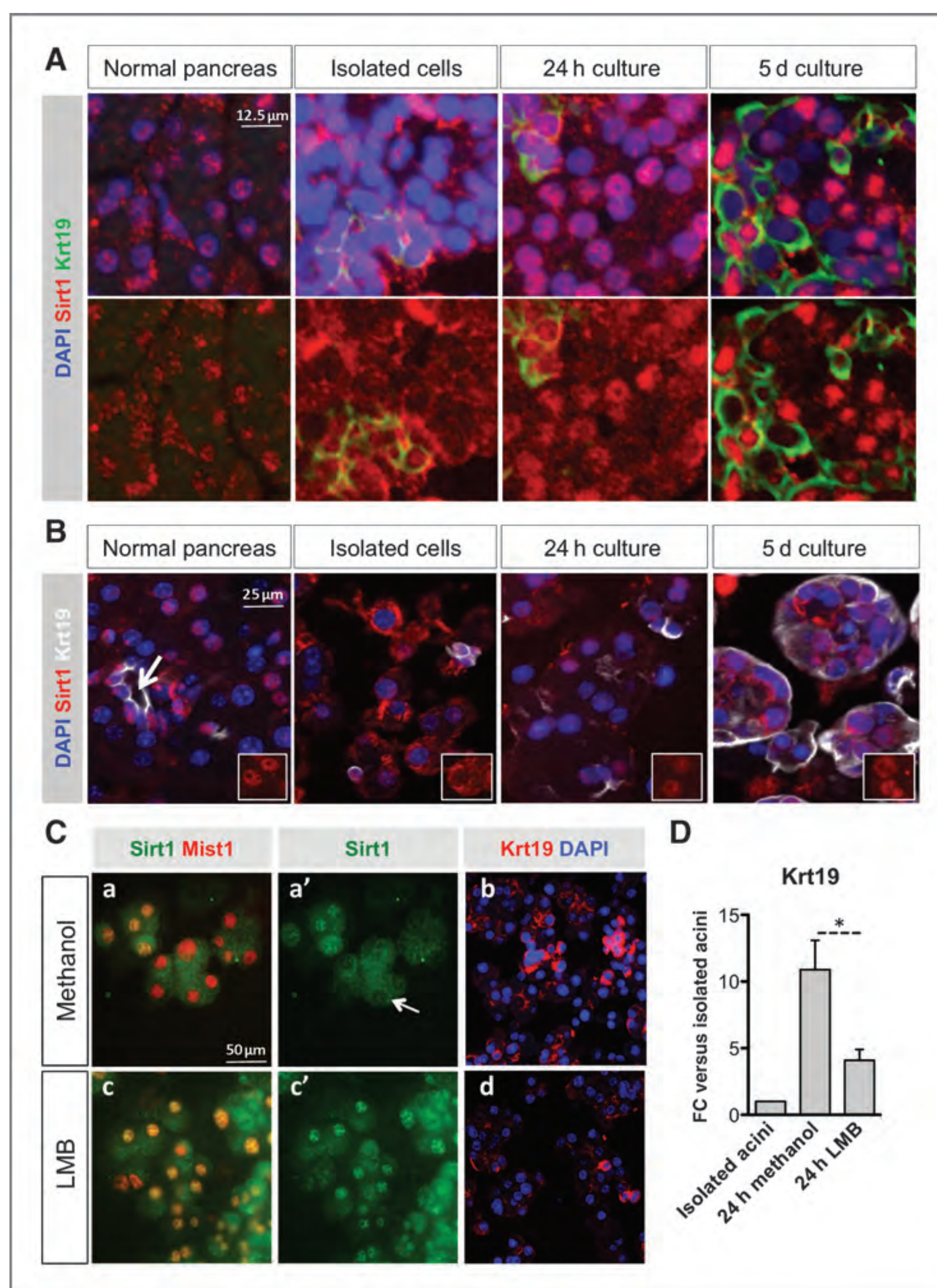


Figure 2. Sirt1 nuclear-to-cytoplasmic shuttling in ADM. A, IF detection of Sirt1 and the ductal marker Krt19 in normal human pancreas and in the *in vitro* model of ADM. B, same as A here in mouse tissue and cells. The inset shows the nuclear-to-cytoplasmic shuttling of Sirt1 in detail. The arrow points at a Krt19-expressing duct in normal pancreas. C, IF detection of Sirt1 and Krt19 in acinar cells treated with the nuclear export inhibitor LMB (c and d) or its solvent methanol (a and b). The arrow points to absence of Sirt1 in the nucleus of cells under control methanol conditions. D, fold change (FC) in mRNA expression of *Krt19* in the conditions of C, that is, cultured acini with LMB or its solvent relative to isolated cells ($n = 5$–$6$; *, $P < 0.05$).

previous study (23). This Sirt1 is an inactive mutant form and Sirt1 expression is also strongly suppressed (Supplementary Fig. S4A and S4B). Whereas there is less *Pancreatic transcription factor-1a* (*Ptf1a*), no significant changes occur in the expression of the acinar genes *Amylase 2 (Amy2), Elastase 1 (Ela1), Carboxypeptidase A1 (CpA1), Chymotrypsin B1 (Ctrb1), or the recombinant signal binding protein for immunoglobulin kappa J-region-like (Rbpjl)* (Fig. 3B, control), suggesting that absence of Sirt1 in unstressed conditions does not affect acinar differentiation. However, upon acute caerulein pancreatitis (Fig. 3A), the role of Sirt1 becomes apparent, with the pancreata from the Pdx1-Cre;Sirt1$^{ex4lox/lox}$ mice showing a more profound ADM (Fig. 3A and B), characterized by a higher suppression of acinar enzymes and of the transcription factors Ptf1a and Rbpjl. Similar results were obtained using the *in vitro* ADM model (Supplementary Fig. S4C). In the Pdx1-Cre;Sirt1$^{ex4lox/lox}$ mice, the ADM was also more persistent with suppression of acinar markers 8 days post-caerulein. At this time point, the pancreatic tissue from control mice had almost completely recovered (with <10% of affected areas in tissue sections), whereas ADM lesions were still manifest in Pdx1-Cre;Sirt1$^{ex4lox/lox}$ mice (3 of 5 animals with >50% of affected areas in tissue sections). We also noticed a reduction in pancreas volume in the Pdx1-Cre;Sirt1$^{ex4lox/lox}$ (not shown). These



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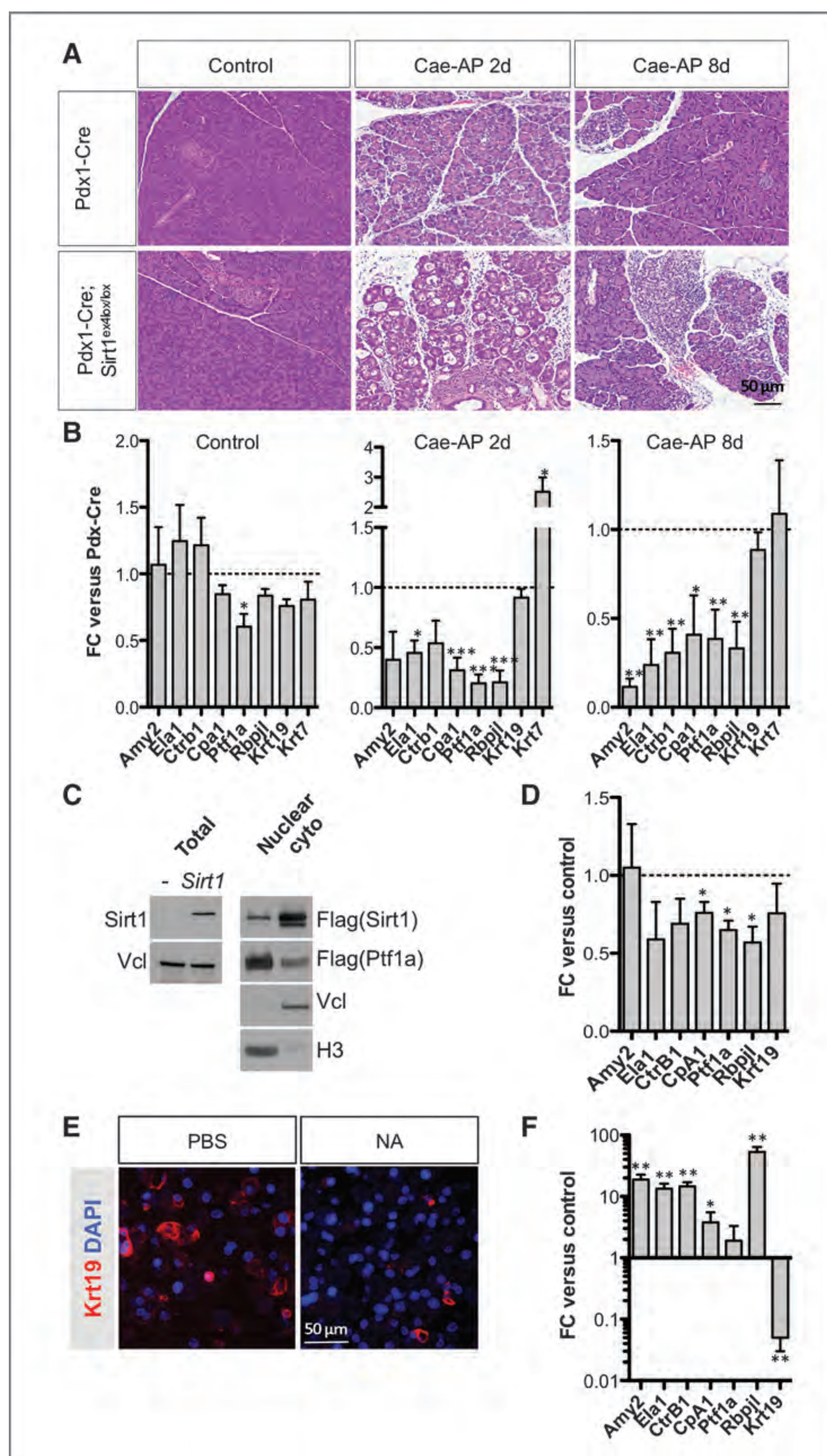


Figure 3. Modulation of Sirt1 expression and activity in ADM. A, hematoxylin and eosin staining of representative tissue sections from caerulein acute pancreatitis (2, 8 days and controls) in Pdx1-Cre; Sirt1$^{ex4lox/lox}$ mice and Pdx1-Cre only mice. B, fold change (FC) in mRNA expression of acinar genes *Amy2, Ela1, CtrB1, CpA1, Ptf1a*, and *Rbpjl* and ductal genes (*Krt19* and *Krt7*) in the same conditions as A comparing Pdx1-Cre;Sirt1$^{ex4lox/lox}$ mice with Pdx1-Cre only mice ($n = 5$; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$). C, Western blotting for Sirt1 using a total protein extract from 266-6 cells stably infected with a vector expressing human Sirt1 (84% homology with mouse) or a control with empty vector (–). Protein input was assessed with vinculin (Vcl; left). Western blotting using fractionated samples of the Sirt1-overexpressing cells to assess Sirt1 and Ptf1a. Vinculin and histone H3 are used as controls for fractionation (right). D, fold change (FC) in mRNA expression of acinar genes in 266-6 cells stably infected with Sirt1 relative to controls ($n = 3$; *, $P < 0.05$). E, IF detection of Krt19 in acinar cells treated with nicotinamide (NA) or its solvent PBS. F, fold change (FC) in mRNA expression of acinar genes in cultured acini with NA, relative to controls ($n = 5$–6; *, $P < 0.05$; **, $P < 0.005$). Dotted lines in B, D, and F refer to control levels that equal 1.



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results underscore that, under stress conditions, absence of Sirt1 enhances the ADM.

To evaluate whether increased Sirt1 expression also had effects on acinar gene expression and ADM, we analyzed a Sirt1 transgenic (SirtTg) mouse strain (24) that expressed increased Sirt1 levels from its endogenous promoter ubiquitously, including in acinar cells (Supplementary Fig. S5A), to evaluate whether increased Sirt1 expression had effects on the acinar gene expression. We did not detect significant differences (Supplementary Fig. S5B**)** either in unstressed or upon pancreatitis/ADM conditions. We note that the Sirt1 overexpression in acinar cells was modest (~2-fold), in line with previous study (24).

To further study Sirt1 overexpression and address the question whether Sirt1 accumulation in the cytoplasm of acinar cells has a contribution to ADM, we analyzed whether acinar 266-6 cells with stable and robust overexpression of Sirt1 mostly confined to the cytoplasm (Fig. 3C) had different features. We found consistent suppression of CpA1, Ptf1a, and Rbpjl (Fig. 3D).

Finally, we wanted to explore whether pharmacologic interference could affect ADM. We used a Sirt1 inhibitor nicotinamide (NA) in the ADM *in vitro* model. Nicotinamide treatment during the first 24 hours, when Sirt1 was predominantly cytoplasmic, resulted in a suppression of Krt19 induction, noted by IF and reverse transcription-quantitative PCR (RT-qPCR) (Fig. 3E and F). In addition, higher expression of the acinar cell–specific markers *Amy2, Ela1, Ctrb1, CpA1*, and *Rbpjl* was maintained (Fig. 3F). Similar ADM repression was observed in preliminary experiments with human acinar cells exposed to nicotinamide (Supplementary Fig. S6A and S6B).

In conclusion, loss of nuclear Sirt1 and cytoplasmic accumulation both contribute to ADM. Inhibition of the shuttling activity of Sirt1 during ADM restrains the process.

### Sirt1 deacetylates β-catenin and Ptf1a

Sirt1 can deacetylate β-catenin, a posttranslational modification that impairs its transcriptional activity (25), and modulates Wnt/β-catenin signaling, as in colon cancer (26). Loss of β-catenin signaling is decisive in installing persistent ADM and impairs acinar cell regeneration (21).

In acini, β-catenin is associated with the plasma membrane (Fig. 4A). A cytoplasmic localization of β-catenin has been reported in ADM (21, 27, 28) and correlates well with those conditions in which we showed a predominant cytoplasmic Sirt1. Therefore, we investigated a possible relation of β-catenin and Sirt1 in ADM.

First, we analyzed the cellular localization of β-catenin: in isolated acini, preferential membrane and occasional nuclear staining were seen. A cytoplasmic distribution predominated at 24 hours with a redistribution to the membrane by 5 days of culture (Fig. 4A). This transient cytoplasmic staining was not observed in presence of nicotinamide (Fig. 4B). In Western blotting, 2 bands were detected: The higher band likely results from posttranslational modifications (see below) and persisted upon treatment with nicotinamide, whereas the lower band was induced in controls (Fig. 4C). In Cae-AP *in vivo,* we found a similar pattern of β-catenin in immunostaining (Supplementary Fig. S1B and S1C) and in Western blot analysis (not shown).

We then used AR42J-B13 cells to further investigate the functional interaction of Sirt1 with β-catenin in an acinar cell context. We chose these cells because Sirt1 was predominantly localized in the cytoplasm (Supplementary Fig. S7A). The 2 reported isoforms of Sirt1 were detected (not shown). Sirt1 co-localized and interacted with β-catenin, as shown by IF, the Duolink assay and co-immunopreciptitation (IP; Supplementary Fig. S7A and S7B). Using co-IP, we detected the Sirt1 isoform that has the highest deacetylase activity (29). As of β-catenin, 2 bands were detected (Fig. 4E), consistent with findings by others (30). By co-IP, we showed that the upper β-catenin band corresponded to an acetylated form (Fig. 4E). Exposure to resveratrol to stimulate Sirt1 activity (31) resulted in a 3.2-fold increased density of the lower deacetylated β-catenin band ($P < 0.05$, $n = 4$), specifically within the cytoplasm (Supplementary Fig. S7C). Of note, the upper band was maintained when nicotinamide was applied to the primary culture model (Fig. 4C).

We then examined whether our observations are related to Wnt/β-catenin signaling. *Axin2*, a Wnt/β-catenin target gene, was induced upon acinar cell isolation but was highly reduced at 24 hours in control (PBS) ADM cultures (Fig. 4D). This paralleled the IF data where membrane-associated β-catenin and accumulation of β-catenin in the nucleus were only observed in isolated acini (Fig. 4A). With nicotinamide, the expression of *Axin2* was maintained at higher levels (Fig. 4D). We confirmed this effect of nicotinamide on Wnt/β-catenin signaling by comparing a larger panel of targets and regulators of the pathway (Supplementary Fig. S8A and S8B).

The nuclear co-localization of Sirt1 and Ptf1a in normal acini (Fig. 5A), the changes in subcellular localization of Sirt1, and the changes in digestive enzyme levels in ADM suggested that Sirt1 might contribute to regulate the function of PTF1, the master regulator of the acinar program. PTF1 is a transcription factor complex composed of Ptf1a, Rbpjl, and a class A bHLH protein (32). P300/CBP-associated factor (pCAF) is associated to it, acetylates Ptf1a, and ensures high transcriptional activity of Ptf1a (33). We undertook chromatin immunopreciptitation (ChIP) experiments to verify whether Sirt1 localized together with Ptf1a in the PTF1-binding sites of acinar gene promoters. *CpA1, CtrB1, Ela1, Ptf1a*, and *Rbpjl* promoter areas were indeed 3- to 30-fold enriched in anti-Sirt1 ChIPs (Fig. 5B). To assess whether Sirt1 and Ptf1a interact and impact on the acetylation state, Flag-pCAF, Flag-Ptf1a, Flag-Sirt1, and Flag-Sirt1* [a mutant Sirt1 in which a critical histidine in the deacetylase domain has been replaced by a tyrosine residue (H363Y)] were transfected in HEK293 cells and assayed by co-IP. The results confirmed that Ptf1a became acetylated upon co-transfection of pCAF (as reported in ref. 33), and there is loss of acetylation of Ptf1a when co-transfected with Sirt1, but not when co-transfected with the inactive Sirt1* (Fig. 5C). There were no effects on Rbpjl (not shown).

Together, our findings showed altered acetylation of the Sirt1 target β-catenin in the context of ADM and thereby interference with β-catenin/Wnt signaling. Furthermore, Sirt1



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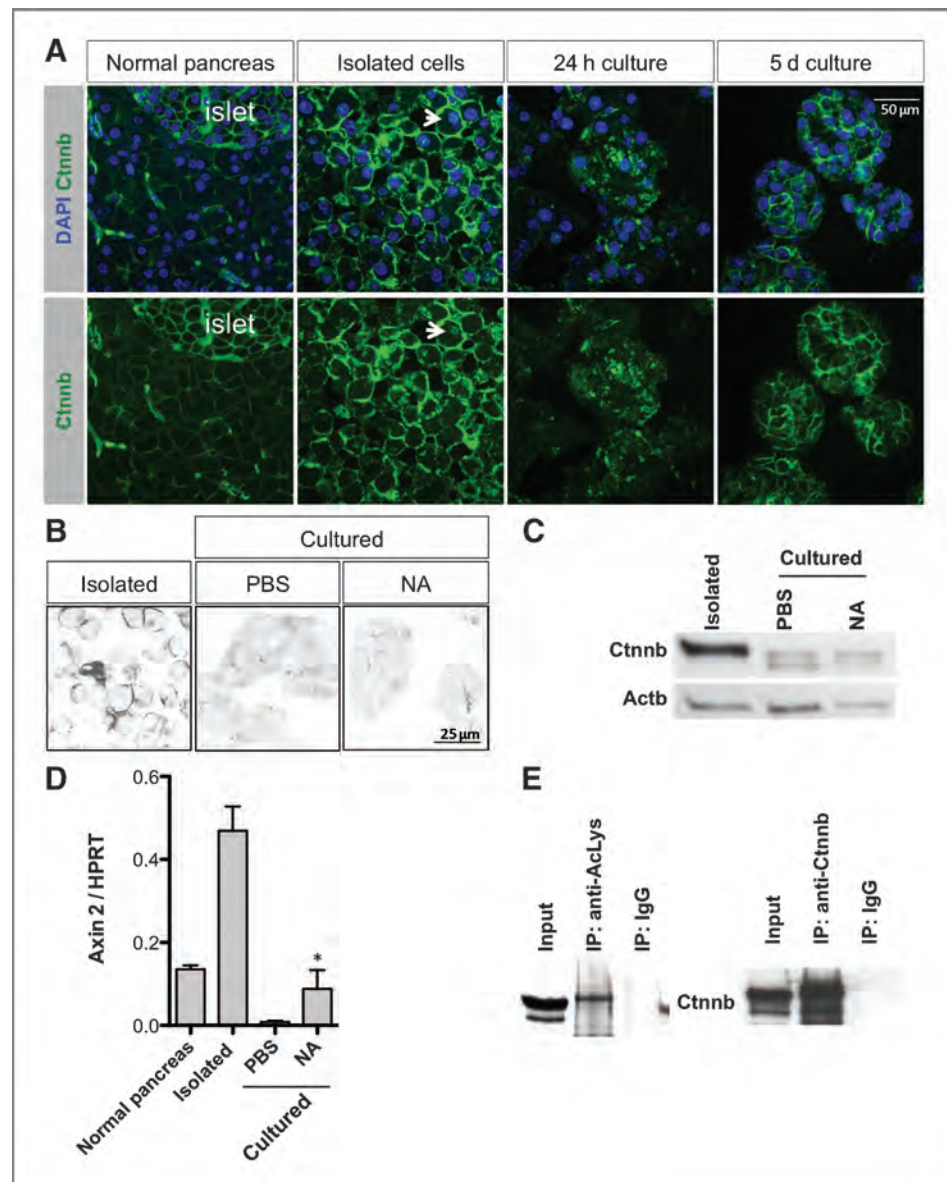


Figure 4. Targets of Sirt1 in acinar cells: β-catenin. A, IF detection of β-catenin (Ctnnb) in normal mouse pancreas and in different stages of ADM cultures (isolated acini, 24h and d5). The white arrow points at nuclear β-catenin staining. B, IF detection of β-catenin comparing isolated acini and acini cultured with nicotinamide (NA) or its solvent PBS. C, Western blotting for β-catenin (Ctnnb) and β-actin (Actb) in isolated acini or acinar cells cultured for 24 hours with NA or PBS. D, mRNA expression levels of *Axin2* (a direct target of Wnt/β-catenin signaling) in normal pancreas, isolated acini, and acinar cells cultured with NA or PBS ($n = 5$–$12$; $^*$, $P < 0.05$). E, co-IP of acetyl lysine (AcLys) and β-catenin (Ctnnb) in acinar AR42J-B13 cells. Results are shown for one representative experiment.

could deacetylate Ptf1a, the critical component of PTF1, and Sirt1 associated with PTF1-dependent gene promoters. These observations underpinned that Sirt1 can regulate acinar cell differentiation and have a role in ADM.

**Role of Sirt1 in advanced pancreatic tumors**

Subsequent to our investigations in metaplasia, we studied Sirt1 in human PDAC. Using Illumina expression microarray data from (34), we found that *Sirt1* in PDAC tissue does not differ from normal pancreas ($P = 0.23$). Immunohistochemistry (IHC) resulted in variable detection levels and patterns in normal and cancer tissue, again without apparent differences (not shown).

We studied the effect of genetic inhibition of Sirt1 expression in Panc1 and Panc10.05 cells with siRNAs. Knockdown of Sirt1 protein was confirmed up to 120 hours after transfection (Fig. 6A), and increased acetylation of p53 was detected, in accordance with p53 being the best described target of Sirt1 (23). No difference was noted in β-catenin or Axin2 expression (Fig. 6A, not shown). Up to 50% inhibition of cell viability was showed (Fig. 6B). In addition, we treated 4 PDAC cell lines with the Sirt1/2 inhibitor Tenovin-6. This reduced the percentage of viable HPAC, MiaPaca2, and Panc10.05 cells in a concentration-dependent manner. However, Panc1 cells were much less sensitive (Fig. 6C).

The observed differences in sensitivity to Tenovin-6 led us to hypothesize that the relative expression levels of Sirt1/Dbc1 determine the sensitivity. Sirt1/Dbc1 protein expression, as measured in Western blotting, did indeed correlate with the $IC_{50}$ for Tenovin-6 ($R = 0.92$, $P < 0.05$; Fig. 6D). In fact, Dbc1 expression is different among PDAC samples (Fig. 6E and F). Compared with high Dbc1 intensity in normal tissue, the



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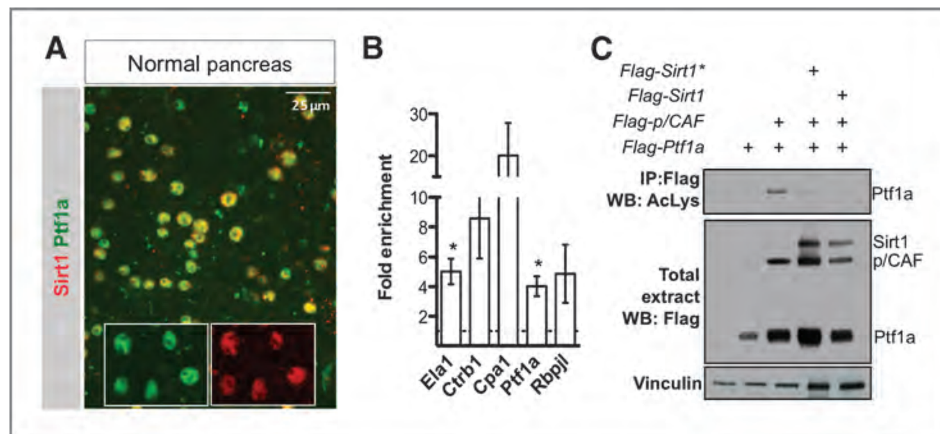


Figure 5. Targets of Sirt1 in acinar cells: Ptf1a. A, IF detection of Sirt1 and Ptf1a in normal mouse pancreas. Both channels are shown separately in the insets. B, Sirt1-ChIP analysis in acinar cells. Fold enrichment of PTF1-containing promoter areas of acinar genes over controls (dotted line refers to controls that equal 1; $n = 3$; *, $P < 0.05$). C, co-IP in HEK293 cells transfected with Flag-pCAF, Flag-Ptf1a, Flag-Sirt1, and/or mutant Flag-Sirt1* and immunoblotted for acetyl-lysine (AcLys) and Flag. Protein input was assessed with vinculin.

fraction of samples with low or medium intensity was significantly higher in PDAC and about 10% were negative (Supplementary Fig. S9A). The percentage of positive nuclei ranged between 3% and 100% in PDAC compared with 50% to 100% in controls (Supplementary Fig. S9B). Dbc1 staining in chronic pancreatitis did not differ from controls (Fig. 6A; Supplementary Fig. S9A and S9B). In our Illumina expression microarray, we also found an overall decrease in *Dbc1* expression in PDAC samples compared with samples of adjacent normal pancreas (fold change = 0.6, $P = 0.0007$).

In conclusion, 3 of 4 PDAC cell lines that we tested are sensitive to Tenovin-6 and this highly correlated with their Sirt1/Dbc1 levels.

## Discussion

Our analyses show that Sirt1 is co-localized with Dbc1 in the nucleus of normal exocrine acinar cells. We find that this co-localization is disturbed in ADM, that is, Sirt1 shifts out of the nucleus into the cytoplasm. This was a general observation in 4 different experimental models of ADM, including mouse and human, regardless of the different types and origins of ADM (19). Dissociation of Sirt1 from its inhibitor activates Sirt1 (13, 35). Furthermore, altered intracellular localization likely changes the interactions with target proteins. Other studies have shown that intracellular Sirt1 localization changes in response to physiologic and pathologic stimuli resulting in altered cell differentiation and different roles during multistage carcinogenesis (22, 36, 37). The functional impact of these changes in Sirt1 in pancreas was explored in this study.

We manipulated the intracellular shuttling and the activity of Sirt1 in acini that undergo ADM. In the presence of LMB, when more nuclear Sirt1 is retained, we observed a lesser induction of Krt19, a prominent hallmark of ADM (2, 15). This indicated inhibition of ADM to a certain extent. We did not prevent the drop in acinar gene expression except when directly inhibiting the activity of Sirt1 with nicotinamide at the time of its cytoplasmic localization. This resulted in the partial preservation of typical acinar gene expression, an extension of our previous observations (4). Preliminary experiments with human cultured acinar cells showed similar findings reinforcing that our observations are relevant in human. It needs to be appreciated that the models used here (mouse models and human exocrine cultures) provide insights into initiating mechanisms that cannot be gathered from clinical samples usually collected at advanced disease, that is, chronic pancreatitis or pancreatic cancer. Nicotinamide is used in clinical trials and more potent and Sirt1-specific drugs that mimic Dbc1 are under development. These may interfere with ADM in pancreatitis and ultimately prevent development of PDAC.

Absence of functional Sirt1 in unstressed pancreas did not seem to impinge on acinar differentiation, likely because of the inhibited state of Sirt1 in normal pancreas or compensatory mechanisms. Under conditions of pancreatitis, however, it becomes clear that lack of nuclear Sirt1 aggravates the ADM. On the other hand, the increased cytoplasmic Sirt1 can also contribute to ADM. We indeed found repression of acinar genes in Sirt1-overexpressing 266-6 cells where expression was mainly in the cytoplasm. The effect was modest perhaps because our experiment was conducted in this cell line that has a less differentiated phenotype than mature acinar cells.

Next, we investigated the mechanisms through which Sirt1 could affect acinar cell differentiation in ADM. β-Catenin has an important role in embryonic acinar cell differentiation and proliferation (38, 39). Its abnormal expression in the cytoplasm in early stages of ADM (and present results; refs. 18, 21, 27) coincident with altered Sirt1 localization suggested a functional link between both proteins. We hypothesized that β-catenin could be a target for deacetylation by Sirt1 in ADM as in colon cancer (26). Our experiments show that in an acinar cell context both proteins can co-localize, interact, and that there is a relation between the acetylation of β-catenin and modulation of Sirt1 activity (nicotinamide and resveratrol). In addition, we discovered that in pancreatic acini Sirt1 is a novel regulator of Wnt/β-catenin signaling that is induced upon acinar cell isolation but rapidly lost in culture. Consequently, the acinar cells dedifferentiate permanently, in agreement with previous observations (21). We find that application of a sirtuin



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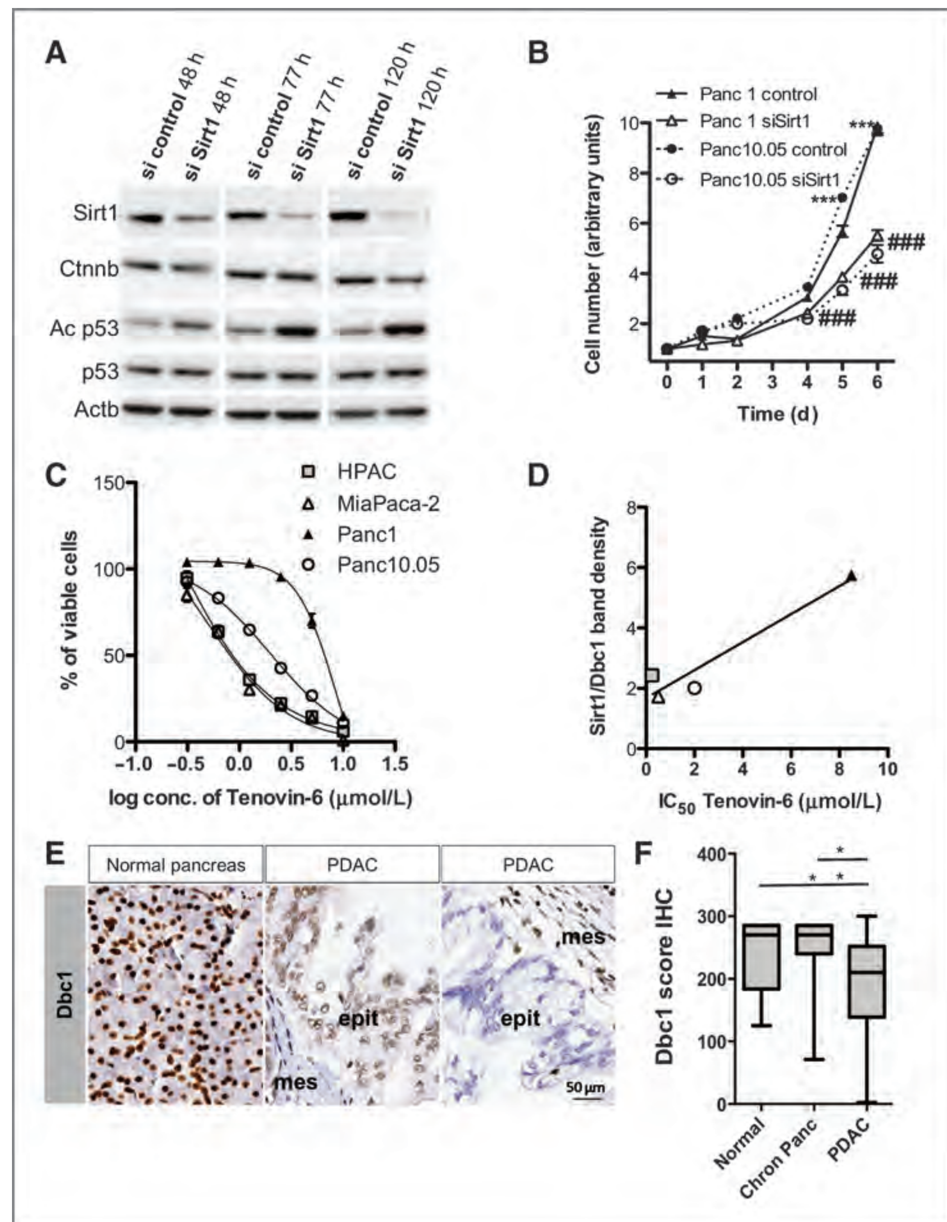


Figure 6. Sirt1 expression and function in PDAC. A, Western blotting for Sirt1, β-catenin (Ctnnb), acetylated (Ac), and total p53 in PDAC cells treated with siRNA for Sirt1 or a scrambled sequence at different time points after transfection. A representative result is shown for Panc1. B, viable cell number as a function of time and relative to day 0 in Panc1 and Panc10.05 cells transfected with siRNA for Sirt1 ($n = 6$; ***, $P < 0.0001$ in Panc1 cells; ###, $P < 0.0001$ in Panc10.05 cells vs. the control). C, percentage of viable cells in function of the concentration of Tenovin-6 (plotted on a logarithmic scale) represented for 4 PDAC cell lines ($n = 12$). D, correlation plot for Sirt1/Dbc1 protein band density measured in Western blots (not shown) versus $IC_{50}$ for Tenovin-6 in the 4 PDAC cell lines used in C. E, IHC for Dbc1 in a tissue microarrays of human pancreas. A normal pancreas and 2 different PDAC tumors are shown. We distinguish epithelium (epit) and mesenchyme (mes). F, Dbc1 IHC scoring based on combined intensity and percentage positive nuclei in the epithelium. Results are displayed for normal pancreas, chronic pancreatitis and PDAC ($n = 14$–77; *, $P < 0.05$; **, $P < 0.001$).

inhibitor resulted in maintenance of Wnt/β-catenin signaling and more membrane-bound β-catenin. However, effects of β-catenin separate from Wnt signaling might also be involved (40). Ras and Notch signaling (21, 41), pathways that are activated in ADM and relevant to carcinogenesis (18, 21, 41, 42), can also regulate β-catenin. As such, it is worth investigating if/how Ras and Notch signaling impinge on Sirt1 activity and vice versa.

The effects on acinar cell differentiation also pointed to a putative effect on the PTF1 complex, its major regulator. Ptf1a is a critical component of PTF1 (43) whose acetylation by pCAF is essential for high digestive enzyme gene expression (33). The present study adds that Sirt1 and Ptf1a proteins co-localize in acinar cell nuclei at the PTF1-binding sites of acinar gene promoters and that Sirt1 can deacetylate Ptf1a. We propose that in the normal pancreas Dbc1 balances Sirt1 activity and acinar cells remain differentiated. Dbc1 does indeed inhibit the activity of Sirt1 in the pancreas (35). Ptf1a can undergo cytoplasmic translocation in ADM (44, 45), similar to what we observe for Sirt1, making a deacetylation event of Ptf1a more likely. Of note, Ptf1a might also be affected by the action of Sirt1 on pCAF that on its turn can be deacetylated by Sirt1 (46).

Finally, we analyzed Sirt1 in PDACs. We extended the observations of Zhao and colleagues (11) and showed that Tenovin-6 or RNA interference for Sirt1 resulted in loss of PDAC cell viability. The $IC_{50}$ values for Tenovin-6 were in line with what has been reported in other tumor cells (47, 48). In contrast to the study by Zhao and colleagues, we did not detect differences in Sirt1 expression in PDAC



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compared with normal tissue. However, we found a decrease of Dbc1 in PDAC tissues, including a subset with undetectable expression. Loss of Dbc1 has been reported in breast, colon, and lung cancer (12). We propose that variation in Dbc1 is relevant in PDAC as Dbc1 directly determines Sirt1 activity (12, 13, 49). The recent finding that c-myc activates Sirt1, and as such favors tumor growth, by sequestering Dbc1 from Sirt1 supports this hypothesis (50). We applied an assay for quantifying Sirt1 activity (13) but failed to get Sirt1 specific results for our pancreatic tissue and cell extracts (not shown). In addition, we found that PDAC cell lines respond variably to Tenovin-6 and that this correlates with the levels of Sirt1/Dbc1. Our data therefore suggest that Dbc1 can be a biomarker for those pancreatic tumors that benefit from Sirt1-inhibitory drugs.

We did not investigate Sirt1 target genes in PDAC cells, but preliminary data point out that there is no effect on β-catenin (Fig. 6A). A role for p53 (Fig. 6A), the best-characterized target of Sirt1, was beyond the scope of our study.

In conclusion, this is the first study that examines the role of Sirt1 on differentiation and tumor maintenance in the exocrine pancreas, contributing to our understanding of those mechanisms that could be harnessed in therapeutic control.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** E. Wauters, A.V. Pinho, A. Mawson, J. Wu, M.J. Cowley, T. Liu, F.X. Real, I. Rooman
**Writing, review, and/or revision of the manuscript:** V.J.S.-A. Lobo, A.V. Pinho, A. Mawson, T. Liu, L. Bouwens, F.X. Real, A.V. Biankin, I. Rooman, E. Wauters, A.V. Pinho
**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** R.L. Sutherland, A.V. Biankin, I. Rooman
**Study supervision:** R.L. Sutherland, L. Bouwens, I. Rooman

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## References

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012;62:10–29.
2. Rooman I, Real FX. Pancreatic ductal adenocarcinoma and acinar cells: a matter of differentiation and development? Gut 2012;61:449–58.
3. Perez-Mancera PA, Guerra C, Barbacid M, Tuveson DA. What we have learned about pancreatic cancer from mouse models. Gastroenterology 2012;142:1079–92.
4. Rooman I, Heremans Y, Heimberg H, Bouwens L. Modulation of rat pancreatic acinoductal transdifferentiation and expression of PDX-1 in vitro. Diabetologia 2000;43:907–14.
5. Michan S, Sinclair D. Sirtuins in mammals: insights into their biological function. Biochem J 2007;404:1–13.
6. Herranz D, Serrano M. SIRT1: recent lessons from mouse models. Nat Rev Cancer 2010;10:819–23.
7. Bosch-Presegue L, Vaquero A. The dual role of sirtuins in cancer. Genes Cancer 2011;2:648–62.
8. Lee JH, Song MY, Song EK, Kim EK, Moon WS, Han MK, et al. Overexpression of SIRT1 protects pancreatic beta-cells against cytokine toxicity by suppressing the nuclear factor-kappaB signaling pathway. Diabetes 2009;58:344–51.
9. Bordone L, Motta MC, Picard F, Robinson A, Jhala US, Apfeld J, et al. Sirt1 regulates insulin secretion by repressing UCP2 in pancreatic beta cells. PLoS Biol 2006;4:e31.
10. Bastien-Dionne PO, Valenti L, Kon N, Gu W, Buteau J. Glucagon-like peptide 1 inhibits the sirtuin deacetylase SirT1 to stimulate pancreatic beta-cell mass expansion. Diabetes 2011;60:3217–22.
11. Zhao G, Cui J, Zhang JG, Qin Q, Chen Q, Yin T, et al. SIRT1 RNAi knockdown induces apoptosis and senescence, inhibits invasion and enhances chemosensitivity in pancreatic cancer cells. Gene Ther 2011;18:920–8.
12. Kim JE, Chen J, Lou Z. p30 DBC is a potential regulator of tumorigenesis. Cell Cycle 2009;8:2932–5.
13. Nin V, Escande C, Chini CC, Giri S, Camacho-Pereira J, Matalonga J, et al. Role of Deleted in Breast Cancer 1 (DBC1) protein in SIRT1 deacetylase activation induced by protein kinase A and AMP-activated protein kinase. J Biol Chem 2012;287:23489–501.
14. Li L, Wang L, Wang Z, Ho Y, McDonald T, Holyoake TL, et al. Activation of p53 by SIRT1 inhibition enhances elimination of CML leukemia stem cells in combination with imatinib. Cancer Cell 2012;21:266–81.
15. Pinho AV, Rooman I, Reichert M, De Medts N, Bouwens L, Rustgi AK, et al. Adult pancreatic acinar cells dedifferentiate to an embryonic progenitor phenotype with concomitant activation of a senescence programme that is present in chronic pancreatitis. Gut 2011;60:958–66.
16. Houbracken I, De Waele E, Lardon J, Ling Z, Heimberg H, Rooman I, et al. Lineage tracing evidence for transdifferentiation of acinar to duct cells and plasticity of human pancreas. Gastroenterology 2011;141:731–41.
17. Xu X, D'Hoker J, Stange G, Bonne S, De Leu N, Xiao X, et al. Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. Cell 2008;132:197–207.
18. Jensen JN, Cameron E, Garay MV, Starkey TW, Gianani R, Jensen J. Recapitulation of elements of embryonic development in adult mouse pancreatic regeneration. Gastroenterology 2005;128:728–41.
19. Strobel O, Dor Y, Alsina J, Stirman A, Lauwers G, Trainor A, et al. In vivo lineage tracing defines the role of acinar-to-ductal transdifferentiation in inflammatory ductal metaplasia. Gastroenterology 2007;133:1999–2009.
20. Hong SM, Heaphy CM, Shi C, Eo SH, Cho H, Meeker AK, et al. Telomeres are shortened in acinar-to-ductal metaplasia lesions



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associated with pancreatic intraepithelial neoplasia but not in isolated acinar-to-ductal metaplasias. Mod Pathol 2011;24:256–66.

21. Morris JPt, Cano DA, Sekine S, Wang SC, Hebrok M. Beta-catenin blocks Kras-dependent reprogramming of acini into pancreatic cancer precursor lesions in mice. J Clin Invest 2010;120:508–20.
22. Tanno M, Sakamoto J, Miura T, Shimamoto K, Horio Y. Nucleocytoplasmic shuttling of the NAD+-dependent histone deacetylase SIRT1. J Biol Chem 2007;282:6823–32.
23. Cheng H-L, Mostoslavsky R, Saito Si, Manis JP, Gu Y, Patel P, et al. Developmental defects and p53 hyperacetylation in Sir2 homolog (SIRT1)-deficient mice. Proc Natl Acad Sci U S A 2003;100: 10794–9.
24. Pfluger PT, Herranz D, Velasco-Miguel S, Serrano M, Tschop MH. Sirt1 protects against high-fat diet-induced metabolic damage. Proc Natl Acad Sci U S A 2008;105:9793–8.
25. Ge X, Jin Q, Zhang F, Yan T, Zhai Q. PCAF acetylates {beta}-catenin and improves its stability. Mol Biol Cell 2009;20:419–27.
26. Firestein R, Blander G, Michan S, Oberdoerffer P, Ogino S, Campbell J, et al. The SIRT1 deacetylase suppresses intestinal tumorigenesis and colon cancer growth. PLoS One 2008;3:e2020.
27. Minami K, Okano H, Okumachi A, Seino S. Role of cadherin-mediated cell-cell adhesion in pancreatic exocrine-to-endocrine transdifferentiation. J Biol Chem 2008;283:13753–61.
28. Lerch MM, Lutz MP, Weidenbach H, Muller-Pillasch F, Gress TM, Leser J, et al. Dissociation and reassembly of adherens junctions during experimental acute pancreatitis. Gastroenterology 1997;113: 1355–66.
29. Lynch CJ, Shah ZH, Allison SJ, Ahmed SU, Ford J, Warnock LJ, et al. SIRT1 undergoes alternative splicing in a novel auto-regulatory loop with p53. PLoS One 2010;5:e13502.
30. Wallace K, Marek CJ, Hoppler S, Wright MC. Glucocorticoid-dependent transdifferentiation of pancreatic progenitor cells into hepatocytes is dependent on transient suppression of WNT signalling. J Cell Sci 2010;123:2103–10.
31. Alcain FJ, Villalba JM. Sirtuin activators. Expert Opin Ther Pat 2009; 19:403–14.
32. Masui T, Swift GH, Deering T, Shen C, Coats WS, Long Q, et al. Replacement of Rbpj with Rbpjl in the PTF1 complex controls the final maturation of pancreatic acinar cells. Gastroenterology 2010;139: 270–80.
33. Rodolosse A, Campos M-L, Rooman I, Lichtenstein M, Real FX. p/CAF modulates the activity of the transcription factor p48/Ptf1a involved in pancreatic acinar differentiation. Biochem J 2009;418:463–73.
34. Biankin AV, Waddell N, Kassahn KS, Gingras MC, Muthuswamy LB, Johns AL, et al. Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. Nature 2012;491:399–405.
35. Escande C, Chini CC, Nin V, Dykhouse KM, Novak CM, Levine J, et al. Deleted in breast cancer-1 regulates SIRT1 activity and contributes to high-fat diet-induced liver steatosis in mice. J Clin Invest 2010;120: 545–58.
36. North BJ, Verdin E. Interphase nucleo-cytoplasmic shuttling and localization of SIRT2 during mitosis. PLoS One 2007;2:e784.
37. Song NY, Surh YJ. Janus-faced role of SIRT1 in tumorigenesis. Ann N Y Acad Sci 2012;1271:10–9.
38. Dessimoz J, Grapin-Botton A. Pancreas development and cancer: Wnt/beta-catenin at issue. Cell Cycle 2006;5:7–10.
39. Murtaugh LC, Law AC, Dor Y, Melton DA. Beta-catenin is essential for pancreatic acinar but not islet development. Development 2005;132: 4663–74.
40. Dessimoz J, Bonnard C, Huelsken J, Grapin-Botton A. Pancreas-specific deletion of beta-catenin reveals Wnt-dependent and Wnt-independent functions during development. Curr Biol 2005;15: 1677–83.
41. Siveke JT, Lubeseder-Martellato C, Lee M, Mazur PK, Nakhai H, Radtke F, et al. Notch signaling is required for exocrine regeneration after acute pancreatitis. Gastroenterology 2008;134:544–55.
42. Rooman I, De Medts N, Baeyens L, Lardon J, De Breuck S, Heimberg H, et al. Expression of the Notch signaling pathway and effect on exocrine cell proliferation in adult rat pancreas. Am J Pathol 2006; 169:1206–14.
43. MacDonald RJ, Swift GH, Real FX. Transcriptional control of acinar development and homeostasis. Pro Mol Biol Transl Sci 2010; 97:1–40.
44. Dufresne M, Clerc P, Dieng M, Edir A, Couvelard A, Delisle MB, et al. Id3 modulates cellular localization of bHLH Ptf1-p48 protein. Int J Cancer 2011;129:295–306.
45. Adell T, Gomez-Cuadrado A, Skoudy A, Pettengill OS, Longnecker DS, Real FX. Role of the basic helix-loop-helix transcription factor p48 in the differentiation phenotype of exocrine pancreas cancer cells. Cell Growth Differ 2000;11:137–47.
46. Pediconi N, Guerrieri F, Vossio S, Bruno T, Belloni L, Schinzari V, et al. hSirT1-dependent regulation of the PCAF-E2F1-p73 apoptotic pathway in response to DNA damage. Mol Cell Biol 2009;29:1989–98.
47. Yuan H, Wang Z, Li L, Zhang H, Modi H, Horne D, et al. Activation of stress response gene SIRT1 by BCR-ABL promotes leukemogenesis. Blood 2012;119:1904–14.
48. Marshall GM, Liu PY, Gherardi S, Scarlett CJ, Bedalov A, Xu N, et al. SIRT1 promotes N-Myc oncogenesis through a positive feedback loop involving the effects of MKP3 and ERK on N-Myc protein stability. PLoS Genet 2011;7:e1002135.
49. Yuan J, Luo K, Liu T, Lou Z. Regulation of SIRT1 activity by genotoxic stress. Genes Dev 2012;26:791–6.
50. Menssen A, Hydbring P, Kapelle K, Vervoorts J, Diebold J, Luscher B, et al. The c-MYC oncoprotein, the NAMPT enzyme, the SIRT1-inhibitor DBC1, and the SIRT1 deacetylase form a positive feedback loop. Proc Natl Acad Sci U S A 2012;109:E187–96.



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