Published in final edited form as:

Science. 2019 June 21; 364(6446): 1156–1162. doi:10.1126/science.aaw3145.

The Glycan CA19-9 Promotes Pancreatitis and Pancreatic Cancer in Mice

Dannielle D. Engle^{1,2,13}, Hervé Tiriach^{1,2,14}, Keith D. Rivera¹, Arnaud Pommier^{1,2,15}, Sean Whalen³, Tobiloba E. Oni^{1,2}, Brinda Alagesan^{1,2}, Eun Jung Lee^{1,2}, Melissa A. Yao^{1,2}, Matthew S. Lucito^{1,2}, Benjamin Spielman^{1,2}, Brandon Da Silva^{1,2}, Christina Schoepfer^{1,2}, Kevin Wright^{1,2}, Brianna Creighton^{1,2}, Lauren Afinowicz^{1,2}, Kenneth Yu^{4,5}, Robert Grützmann⁶, Daniela Aust⁷, Phyllis A. Gimotty⁸, Katherine S. Pollard^{3,9}, Ralph H. Hruban¹⁰, Michael G. Goggins^{10,11}, Christian Pilarsky⁶, Youngkyu Park^{1,2}, Darryl J. Pappin¹, Michael A. Hollingsworth¹², David A. Tuveson^{1,2,*}

¹Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA.

²Lustgarten Foundation Pancreatic Cancer Research Laboratory, Cold Spring Harbor, NY 11724, USA.

³Gladstone Institutes, San Francisco, CA 94158, USA.

⁴Rubenstein Center for Pancreatic Cancer Research, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA.

⁵Weill Medical College at Cornell University, New York, NY 10065, USA.

⁶Department of Surgery, Universitätsklinikum Erlangen, Erlangen, Germany.

⁷Institute for Pathology, Universitätsklinikum Dresden, Dresden, Germany.

*Correspondence to: dtuveson@cshl.edu.

Author Contributions. DDE generated and characterized the CA19-9 expressing cells, organoids, and mice, analyzed human tissues, and co-wrote the manuscript. HT assisted with organoid and immunoblotting experiments and immunohistochemistry. AP assisted with EdU and immune cell flow cytometry experiments. KR and DP performed LC MS/MS and analyzed proteomic results. TO performed immunization experiments. BA assisted with KC survival studies. CS, MY, BS, BDS, BC, EL, LA, KW, MSL assisted with animal experiments and organoid generation. PAG assisted with statistical analyses. KSP and SW analyzed RNA-seq data. MGG provided the normal adjacent pancreas and tumor TMA and RHH provided pathology diagnoses. CP, RG and DA collected human pancreatitis serum and developed the pancreatitis TMA with associated clinical data. PG reviewed statistical analyses. YP assisted in the generation of the CA19-9 mouse models and provided oversight on studies involving mice. MAH and DAT conceived the project, directed the experiments and co-wrote the manuscript.

Competing Interests. CSHL owns a pending US provisional patent application directed to the use of CA19-9 antibodies to treat pancreatitis, which was filed on behalf of CSHL under a license from CSHL to MabVax Therapeutics Holdings, Inc. DAT serves on the Scientific Advisory Board of Leap Therapeutics, Surface Oncology, and Bethyl Laboratory, which is not related to the subject matter of this manuscript. DAT also serves on the Board of Scientific Advisors for the NCI, the Scientific Advisory Board of AACR, the Scientific Advisory Council of Stand Up to Cancer, and the Scientific Advisory Committee of the Georg-Speyer-Haus Institute for Tumor Biology and Experimental Therapy. DAT is a distinguished scholar of the Lustgarten Foundation and Director of the Lustgarten Foundation-designated Laboratory of Pancreatic Cancer Research.

Data and Materials Availability. The models created and used herein will be available for distribution upon execution of a materials transfer agreement (DAT). The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE(54) partner repository with the dataset identified PXD008564 and can be accessed (<https://www.ebi.ac.uk/pride/archive/>) using the following reviewer account details: Username: reviewer39667@ebi.ac.uk, Password: tBzKBKAc. The RNA sequencing data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus and will be accessible through GEO Series accession number GSE130854 at time of publication (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE130854>) (55). Data can be accessed currently by reviewers using the token: ubwhsgoofnqlhmj.

⁸Department of Biostatistics, Epidemiology and Informatics, University of Pennsylvania, Philadelphia, PA 19104, USA.

⁹Department of Epidemiology and Biostatistics, Institute for Human Genetics; Quantitative Biology Institute, and Institute for Computational Health Sciences, University of California San Francisco; Chan-Zuckerberg Biohub, San Francisco, CA 94158, USA.

¹⁰Sidney Kimmel Cancer Center, The Sol Goldman Pancreatic Cancer Research Center, Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD 21231, USA

¹¹Department of Medicine, Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD 21231, USA

¹²Eppley Institute, University of Nebraska Medical Center, Omaha, NE 68198, USA. Current Addresses:

¹³The Salk Institute for Biological Studies, La Jolla, CA 92037, USA.

¹⁴Department of Surgery, University of California, San Diego, La Jolla CA 92093, USA.

¹⁵Department of Immunology, Cochin Institute, Paris, France.

Abstract

Glycosylation alterations are indicative of tissue inflammation and neoplasia, but whether these alterations contribute to disease pathogenesis is largely unknown. To study the role of glycan changes in pancreatic disease, we inducibly expressed human *Fucosyltransferase 3* and $\beta 1,3$ -*Galactosyltransferase 5* in mice, reconstituting the glycan sialyl-Lewis ^a/Carbohydrate Antigen 19–9 (CA19–9). Notably, CA19–9 expression in mice resulted in rapid and severe pancreatitis with hyperactivation of epidermal growth factor receptor (EGFR) signaling. Mechanistically, CA19–9 modification of the matricellular protein Fibulin 3 increased its interaction with EGFR, and blockade of Fibulin 3, EGFR, or CA19–9 prevented EGFR hyperactivation in organoids. CA19–9-mediated pancreatitis was reversible and could be suppressed with CA19–9 antibodies. CA19–9 also cooperated with the Kras^{G12D} oncogene to produce aggressive pancreatic cancer. These findings implicate CA19–9 in the etiology of pancreatitis and pancreatic cancer and nominate CA19–9 as a therapeutic target.

Introduction

Pancreatitis, or inflammation of the pancreas, is a painful, recurrent and occasionally lethal medical disorder with limited treatment options. Pancreatitis is a common disease, with 33.74 acute and 9.62 chronic pancreatitis cases per 100,000 people worldwide (1). Pancreatitis accounts for more than 275,000 hospitalizations in the United States per year and the number of hospital admissions has increased by 20% over the past decade (2). The causes of pancreatitis include blockage of the pancreatic duct by gallstones, alcohol and certain drugs that cause acinar cell damage, medical procedures or trauma that damage pancreatic tissue, and autoimmune diseases (2). In approximately one third of cases, the underlying etiology of the pancreatitis is unknown (idiopathic) (3, 4). Most acute

pancreatitis cases will resolve with supportive care, however up to 20% of patients will develop severe tissue damage and will either succumb to multi-organ system failure or suffer from bouts of recurrent disease with markedly diminished quality of life (2–4). Individuals with hereditary acute pancreatitis progress to chronic pancreatitis with a much higher penetrance and furthermore have a 40 to 55% lifetime risk of developing pancreatic cancer (1, 5). Indeed, chronic pancreatitis promotes mutant Kras-mediated development of pancreatic cancer in mice (6).

The glycan CA19–9 is found in the serum of 10–30% of pancreatitis patients, 75% of pancreatic cancer patients, as well as in patients with other gastrointestinal diseases (7–16). CA19–9 elevation is also detected in Pancreatic Intra-epithelial Neoplasms (PanINs), which are precursors to pancreatic ductal adenocarcinoma (PDAC) (17). CA19–9 (sialyl-Lewis^a, sLe^a) is generated by the stepwise addition of sugar moieties to Type 1 precursor chains present on proteins and other molecules, culminating in the α 1,4 linkage of fucose to N-Acetylglucosamine (GlcNAc) (fig. S1A). The FUT3 fucosyltransferase is the only enzyme with the ability to add fucose moieties through an α 1,4 linkage and generate CA19–9. Mice lack this enzyme because *Fut3* is a pseudogene in rodents (18, 19).

To facilitate the discovery of PDAC biomarker candidates, we sought to create a mouse model of PDAC that recapitulated the elevation of CA19–9 observed in human patients. This model would enable prioritization of biomarkers that outperform CA19–9. Furthermore, changes to glycosylation often result in functional consequences. Here, we investigate the role of CA19–9 elevation in mouse and organoid models of pancreatic disease.

Recapitulation of CA19–9 elevation and regulation in cultured mouse PDAC cells

To express CA19–9 in mouse cells, we transduced mouse PDAC cells with human *FUT3*. However, expression of *FUT3* alone was insufficient for CA19–9 production, but did lead to increased levels of Lewis^x antigens following removal of terminal galactose moieties present in rodents, but not humans (Fig. 1A). The generation of the related Lewis^x epitopes suggested that reprogramming of the precursor substrates would be necessary for the production of CA19–9 in pancreatic ductal cells. *β 3GALT5* is required for the production of Type I chain precursors (20), which serve as the precursors for the Lewis^a modification (fig. S1A). Accordingly, expression of both *FUT3* and *β 3GALT5* in mouse PDAC cells led to the cell surface expression of CA19–9 at levels equivalent to those observed in human cancer cell lines (Colo205, Suit2) (Fig. 1B and fig. S1B). Comparable CA19–9 levels were observed in the blood of mice following orthotopic transplantation of the CA19–9 expressing mouse and human cells (fig. S1C).

To determine whether the murine PDAC cell proteins harboring CA19–9 modification are similar to those in human PDAC cells, CA19–9 protein carriers were immunoprecipitated (IP) and identified by mass spectrometry (MS) (fig. S2A and table S1). These analyses identified known CA19–9 protein carriers in the *FUT3* and *β 3GALT5* expressing mouse cells (n=3, FC1199, FC1242, FC1245), including CD44, Lgals3bp, Muc1 and Muc5ac (21–24). The human PDAC cell line, MiaPaCa-2, is CA19–9 negative and therefore we used it as

a control to identify human CA19–9 core proteins in the CA19–9 positive cell lines, Capan2, Suit2, and hM1–2D (fig. S2B and table S2). We compared mouse and human CA19–9 protein carriers and found that an average of 72.3% (95% CI 60.3–84.3%, n=3) of the CA19–9 modified proteins identified in all three human PDAC cell lines were also found in the engineered murine PDAC cell lines. Thus, expression of the human *FUT3* and *β3GALT5* genes in mouse cells largely recapitulates the human CA19–9 carrier profile (Fig. 1C).

CA19–9 elevation in mice leads to acute and chronic pancreatitis

We next generated a mouse model with inducible CA19–9 expression to study the effects of this glycan on pancreatic disease pathogenesis (fig. S2, C to E). Using PDX1-Cre (C), we restricted expression of the transgenes to the pancreas, duodenum, and bile duct. Cre-mediated excision of a LoxP flanked STOP (LSL) cassette enables ubiquitous expression of the reverse Tetracycline transactivator (rtTA3) and mKate2 from the CAGS promoter within the Rosa26 locus (**R^{LSL}**) (25). Addition of Doxycycline (Dox) to the diet activates rtTA, enabling *FUT3*, *β3GALT5* and *eGFP* expression from the TRE promoter in the ColA1 locus (F) (26). In addition, we excised the LSL cassette in the R allele to generate animals with Dox-inducible, whole-body CA19–9 expression (R;F). Surprisingly, both C;R^{LSL};F and R;F Dox-treated mice exhibited highly penetrant histologic signs of pancreatitis, including interstitial edema, lymphocyte infiltration, and collagen deposition (Fig. 2A and fig. S3, A and B). All other non-pancreatic tissues appeared histologically normal in both Dox-treated and untreated models and their littermate controls (figs. S4 to S5). The mice progressed from acute to chronic pancreatitis after 28 days of Dox treatment as demonstrated by the clinical histopathological hallmarks of acinar atrophy, accumulation of metaplastic ductal lesions, and persistent fibroinflammatory disease (fig. S3, A and B, and S6, A and B) (27). Pancreatitis was highly penetrant in both models (Fig. 2B and fig. S6, C and D).

Both mouse models demonstrated elevated serum levels of the pancreatic enzymes amylase and lipase within 24 hours of Dox treatment, but no changes were observed in untreated mice and littermate controls (Fig. 2C and fig. S6, C and D). Patients with acute pancreatitis have elevated serum levels of amylase and lipase, but in the chronic phase of pancreatitis, the levels of these enzymes either normalize or decrease even further. We found in our mouse models that the levels of amylase and lipase began to normalize after 4 weeks of Dox treatment, but both C;R^{LSL};F and R;F mice still exhibited histologic signs of chronic pancreatitis. Expression of eGFP and CA19–9 was detected in the pancreas after Dox treatment of C;R^{LSL};F mice and R;F mice, but they were not detected in untreated or control littermates (Fig. 2A and figs. S3C, S7, and S8). In both mouse models, CA19–9 was predominantly expressed in intralobular, intercalated and metaplastic pancreatic ducts as well as in islet cells (Fig. 2A and figs. S3C, S7, and S8). Both models exhibited elevated CA19–9 levels in the circulation (Fig. 2D and fig. S6, C and D). Secreted CA19–9 was also observed coating eGFP-negative endothelial cells as well as fibroblasts (figs. S7B and S8). E-Selectin is an endogenous receptor of CA19–9 expressed by endothelial cells (28, 29), and may explain the accumulation of CA19–9 within the vasculature. Despite recombination in the acinar compartment, acinar cells were rarely observed to be CA19–9 positive. R;F mice exhibited Dox-dependent expression of CA19–9 and eGFP in all tissues examined whereas

in C;R^{LSL};F mice, expression was limited to the pancreas, duodenum and gall bladder (Fig. 2A and figs. S3C to G, S7 to S11).

We next sought to determine the prevalence of CA19–9 elevation in human pancreatic disease and to compare the CA19–9 tissue expression pattern observed in human patients and the CA19–9 GEMMs. We therefore evaluated CA19–9 levels and expression patterns in patients, including specimens of pancreatic cancer and adjacent normal tissue (n=72) as well as surgically resected chronic pancreatitis samples (n=44) (fig. S12 and S13). CA19–9 was expressed at low levels in normal homeostatic pancreatic ducts and at elevated levels in adjacent reactive ducts that consist of atypical, benign pancreatic ducts that occur even in the absence of substantial inflammation (fig. S12A), pre-invasive carcinomas (PanIN-1A, PanIN-1B) and invasive PDAC specimens (fig. S12, B to D) (17). In chronic pancreatitis specimens, CA19–9 was expressed at high levels in the reactive and metaplastic ducts with more sporadic expression in the centroacinar and acinar compartments (fig. S13, A and B, and table S3). Elevated levels of CA19–9 were detected in the serum of 20% of the chronic pancreatitis patients we examined whereas over 93% of these patients exhibited local elevation of CA19–9 in the resected pancreatic specimens by IHC (n=44) (fig. S13, C and D). Together, these data indicate that CA19–9 elevation is a common feature of chronic pancreatitis and that the CA19–9 expression pattern is similar between the CA19–9 mouse models and human patients.

Severe acute and chronic pancreatitis patients often also exhibit weight loss (30, 31). Therefore, we assessed the body condition and weight of both models. While most C;R^{LSL};F mice maintained their overall health status following Dox treatment, Dox treated R;F mice exhibited significant body weight loss (fig. S14A). In severe cases, the weight loss exceeded 20% and required euthanasia. The observed weight loss was not due to pathology of the stomach, duodenum, or colon, and was not associated with an auto-immune reaction, induction of ER stress in the pancreas, or loss of glycemic control (32) (fig. S14B to D). Pancreatic exocrine insufficiency is often observed in cases of chronic pancreatitis and can cause severe weight loss and destruction of the acinar compartment in patients (31). R;F mice exhibited a significant Dox-dependent decrease in pancreatic mass while no change was observed in C;R^{LSL};F mice and littermate controls following Dox treatment (fig. S14E). In addition, we observed a significant and sustained increase in steatorrhea in R;F mice whereas C;R^{LSL};F mice exhibited a transient increase in fecal triglycerides that normalized within 4 weeks of Dox treatment (fig. S14F). The pancreatic atrophy and steatorrhea in R;F mice was accompanied by a significant reduction of fecal elastase in R;F mice within the first week of Dox treatment (fig. S14G). Together, these data indicate that R;F mice suffer from pancreatic atrophy and exocrine insufficiency. Both the increased penetrance of CA19–9 expression in the Cre-independent R;F model and expression of CA19–9 in other tissues may contribute to the severe pancreatitis observed in this model.

Pancreatitis is associated with an influx of macrophages during the acute phase of pancreatitis, and after progression to a chronic condition, T- and B-cells also infiltrate the pancreas in human patients (33, 34). To determine whether this aspect of human pancreatitis is also found in mouse models of pancreatitis, we examined the immune infiltrate in C;R^{LSL};F mice and the established Cerulein model of acute pancreatitis. Flow cytometry

revealed an influx of immune cells into the pancreas, including recruitment of inflammatory monocytes and macrophages, following induction of pancreatitis (Fig. 2E and figs. S15 to S16A). This result was confirmed by IHC (fig. S16B). Neither model showed significant recruitment of T- or B- cells at the acute pancreatitis time points examined by flow cytometry (1 – 3 days) (fig. S15). Additionally, both R;F and C;R^{LSL};F models contained intrapancreatic T- and B-cells at the chronic phases of pancreatitis as indicated by IHC (fig. S17). Therefore, both the CA19–9 and Cerulein mouse models of pancreatitis exhibited similar immune infiltrates as those found in human pancreatitis patients.

Pancreatic proliferation is a common feature of human pancreatitis (35). Cerulein treated mice exhibited elevation in Ki67 positive nuclei in the infiltrating immune cells as well as in the ductal and acinar compartments. Similarly, both R;F and C;R^{LSL};F mice exhibited increased Ki67 positive nuclei in the immune, acinar, and ductal compartments in the acute phases of pancreatitis, which gradually diminished as the mice progressed to chronic pancreatitis (fig. S18, A to E). Increased EdU incorporation was also observed in the pancreas, including elevation in the immune, ductal, and fibroblast compartments (Fig. 2F and fig. S18F). Together, these data demonstrate that CA19–9 mediated pancreatitis in mice bears similarity to the human disease.

CA19–9 expression results in hyperactivation of epidermal growth factor receptor (EGFR) signaling

To identify the molecular mechanisms underlying the pancreatitis phenotype observed in mice, we focused our studies on the C;R^{LSL};F model. Other researchers have established that EGFR signaling is necessary and sufficient for the induction of pancreatitis and is important for the initiation of pancreatic cancer in mice (36, 37). Accordingly, we found that the levels of tyrosine phosphorylated and activated EGFR were prominently elevated in the pancreatic ducts of C;R^{LSL};F mice following Dox treatment (Fig. 3A). To identify the signaling pathway changes that occur in direct response to CA19–9 expression, we isolated pancreatic ductal organoids from C;R^{LSL};F mice (38). RNA-seq analyses of the C;R^{LSL};F organoids following induction of CA19–9 expression revealed the expected changes in transgene expression (fig. S19, A to F, and table S4). *FUT3* and *β3GALT5* expression levels were comparable between mouse and human ductal organoids (fig. S19D) (39).

Gene Set Enrichment Analysis (GSEA) identified significant changes to 21 pathways in the Hallmarks of Cancer gene sets and 64 pathways annotated in the KEGG pathway gene sets (Fig. 3B and table S4). CA19–9 expression triggered a reduction in expression of genes associated with the Unfolded Protein Response and an increase in expression of genes associated with Chemokine, Hh, JAK/STAT and TGFβ signaling. The latter genes may participate in the activation of the fibroblast compartment and recruitment of the immune infiltrate (e.g. *Il1a*, *Csf1*, *Tnf*). We further explored enrichment of the extracellular matrix (ECM)-Receptor Interaction, ERBB, PI3K and AKT signaling pathways (Fig. 3B). CA19–9 expression in C;R^{LSL};F organoids resulted in elevated EGFR phosphorylation (Y1068, Y1148) and decreased total EGFR, indicating greater flux through this pathway (40) (Fig. 3C and fig. S20, A to B and E to G). The increase in phosphorylated EGFR was

accompanied by increased phosphorylation of FAK (Y397), AKT and ERK1/2 (Fig. 3C and fig. S20F). Co-incident FAK and EGFR activation has been previously reported in three-dimensional, but not monolayer culture models, and may involve integrin-mediated interaction with the ECM (41, 42). EGFR stimulation by EGF was more rapid and robust in organoids that were previously cultured in EGF-free conditions, but was unable to induce FAK phosphorylation (fig. S20, C and D). While the alterations of phosphorylated EGFR, total EGFR, and their ratio were consistent among biological replicates, no consistent changes to total or phosphorylated S6, HER2 and p65 were observed (fig. S20, A and B). These findings were independent of the inclusion of murine EGF in the media of the cultures (fig. S20E) and the level of phospho/total EGFR and downstream effector induction exceeded the increase observed following treatment with additional EGF (fig. S20, B to D).

The glycosylation state of EGFR has been reported to affect its ability to activate and to respond to targeted kinase inhibitors (43, 44). Accordingly, we examined whether EGFR was modified by CA19–9 in mouse organoids, but this was not detected (fig. S20H). To discern whether the CA19–9 dependent activation of EGFR was due to a soluble ligand, we evaluated conditioned medium from CA19–9 expressing organoids and found that it stimulated EGFR phosphorylation in control murine ductal organoids (Fig. 3, D to F). This activity was attenuated by the addition of EGFR-Fc (EGF Trap), further supporting the presence of one or more EGFR ligands (Fig. 3D and F). Addition of a monoclonal antibody to CA19–9 (NS19–9) was also sufficient to block EGFR phosphorylation in C;R^{LSL};F organoids (Fig. 3, D and G). These findings suggested the presence of a CA19–9 modified, secreted EGFR ligand.

EGFR complexes from C;R^{LSL};F organoid lysates were identified by IP/MS (Fig. 4A and table S5). We observed elevated levels of endogenous Fibulin 3 (*Efemp1*, FBLN3) by IP/MS in C;R^{LSL};F, but not control organoids, following Dox treatment (Fig. 4A). FBLN3, a secreted matricellular glycoprotein with five EGF-like domains, has been proposed to be a ligand for EGFR (45). Furthermore, we performed CA19–9 IP/MS from the conditioned media using two different CA19–9 monoclonal antibody clones and identified FBLN3 as a secreted, CA19–9 modified protein (Fig. 4B, fig. S21A, and table S6).

To confirm the glycosylation status of FBLN3, we co-expressed FLAG epitope tagged FBLN3 in the presence or absence of *FUT3* and *β3GALT5* in mouse PDAC cells, and detected CA19–9 modification of secreted FBLN3 (fig. S21B). The mRNA expression of Fibulin family members, and EGFR ligands did not change following Dox treatment of C;R^{LSL};F organoids (fig. S21C). Furthermore, the total protein levels of FBLN3 were unchanged following CA19–9 expression in organoid conditioned media and in mouse plasma *in vivo* (fig. S21D). Secreted FLAG-FBLN3 isolated by immunoprecipitation co-precipitated with EGFR in PDAC cell lysates, consistent with an association of Fbln3 with EGFR (Fig. 4C). Since the association of ectopic FLAG-FBLN3 with EGFR in cell lysates occurs irrespective of its CA19–9 glycosylation status, the finding that the glycosylation status of endogenous FBLN3 increases association with EGFR may reflect the interaction of sialyl-Lewis^a modified FBLN3 with additional extracellular proteins. To determine the role of FBLN3 in the activation of the EGFR pathway following CA19–9 expression, multiple FBLN3 blocking antibodies and shRNA hairpins were utilized and shown to prevent EGFR

phosphorylation and activation of downstream effector pathways in C;R^{LSL};F organoids (Fig. 4, D and E, and fig. S21, E and F).

CA19–9 as a therapeutic target in pancreatitis

We designed a Dox pulse/chase approach to determine if CA19–9 expression is required to maintain pancreatitis. CA19–9-mediated pancreatitis was completely reversed in C;R^{LSL};F mice following a 3-day Dox pulse and 4-day recovery period (fig. S22, A to C). Partial resolution of chronic pancreatitis in the C;R^{LSL};F model (28-day Dox treatment) was also observed after a 14-day recovery period (fig. S22, D and E). In a preventive setting of acute pancreatitis, two antibodies directed against CA19–9 both reduced immune infiltration, ductal metaplasia, and fibrosis *in vivo* (Fig. 5A, and fig. S22, F and G). In addition, decreased release of amylase and lipase into the circulation, and reduced hyperactivation of EGFR was observed *in vivo* (Fig. 5, B and C, and fig. S22, H and I). In an intervention setting of existing acute pancreatitis, we found that two forms of 5B1 significantly reduced secretion of amylase into the circulation with modest normalization of the pancreatic histology (fig. S22, J to L). CA19–9 antibody treatment of existing acute pancreatitis also reduced the levels of phosphorylated EGFR in both the ductal and acinar compartments and decreased recruitment of macrophages (fig. S22, M and N). These data suggest that CA19–9 plays a role in disease pathogenesis and maintenance and that CA19–9 targeted therapy may warrant further therapeutic exploration.

Interestingly, inhibition of EGFR *in vivo* using Erlotinib was not as effective as CA19–9 antibody blockade to mitigate pancreatitis induction in mice (fig. S23, A to F). Indeed, Erlotinib treatment of CA19–9 expressing mice caused severe weight loss, necessitating euthanasia. These effects were unrelated to Erlotinib toxicity in control mice. Although serum levels of amylase and lipase decreased in Erlotinib-treated animals, pancreatic atrophy and acinar cell vacuolization was observed, suggesting that the weight loss was due to increased pancreatitis severity and resulting exocrine insufficiency (fig. S23, B and C). Erlotinib treatment also incompletely blocked phospho-EGFR levels *in vivo* (fig. S22D). Acinar-ductal metaplasia (ADM) can be detected by Sox9 IHC (36) and occurred after 7 days of Dox treatment in the C;R^{LSL};F model (fig. S22E). Although ADM was less apparent in the Erlotinib treated C;R^{LSL};F mice (fig. S22F), lymphocyte infiltration and fibrosis remained unaffected. CA19–9 sequestration is unlikely to interfere with ADM survival mechanisms in the acinar compartment given that these cells are largely CA19–9 negative (46). Therefore, EGFR kinase inhibition alone cannot substitute for CA19–9 blockade, and may be a harmful therapy in the setting of pancreatitis.

Pancreatic tumorigenesis is accelerated by CA19–9-mediated pancreatitis

To determine if CA19–9 expression promoted PDAC, we intercrossed the C;R^{LSL};F alleles with the conditional Kras^{LSL-G12D} allele (K;C;R^{LSL};F) (fig. S24A) (47, 48). CA19–9 expression significantly accelerated pancreatic cancer lethality relative to untreated mice and control littermates (Fig. 6A). When treated with Dox, K;C;R^{LSL};F mice rapidly succumbed to primary and metastatic pancreatic cancer with a median survival of 202 days relative to 460 days in the K;C control cohort and 420 days in the untreated K;C;R^{LSL};F cohort. The

primary tumors were anaplastic with glandular features (Fig. 6B). Widespread metastases were observed in the peritoneum, diaphragm, liver, and lung in multiple Dox treated K;C;R^{LSL};F mice. To better understand the role of CA19–9 in PDAC initiation, we examined the effect of short-term CA19–9 expression on pancreatic transformation. Whereas littermate controls exhibited the expected low burden of mPanIN-1A lesions, CA19–9 expressing animals harbored a high penetrance of cystic and fibroinflammatory disease with abundant mPanIN-1B and occasional mPanIN-2 lesions after 2 weeks of Dox (Fig. 6C) and increased macrophage infiltration relative to the K;C control cohort (fig. S24, B and C). After 4 weeks of Dox, cystic papillary neoplasia and invasive carcinoma could be detected (Fig. 6C). CA19–9 expression was elevated in normal, benign reactive, and metaplastic ducts as well as in mPanIN and PDAC lesions in K;C;R^{LSL};F mice, similar to the human expression pattern (Fig. 6D and fig. S12). Equivalent levels CA19–9 were also observed in human PDA and mouse K;C;R^{LSL};F organoids (fig. S24D). Phosphorylated EGFR (Fig. 6E) was detected at high levels in Dox-treated K;C;R^{LSL};F mice whereas CA19–9 negative K;C mice exhibited low to negative levels of phospho-EGFR.

Discussion

CA19–9 is expressed at low levels in the normal ducts of the pancreas, but becomes elevated in benign reactive, metaplastic, and malignant ducts in humans. It is conceivable that the degree of CA19–9 elevation could impart a way to control the degree of fibroinflammatory response in both pancreatitis and PDAC. Interestingly, mice retain the ability to respond to elevations in CA19–9 despite lacking the ability to express this glycan. The ability of mice to respond to CA19–9 elevation may be due to the existence of similar regulatory mechanisms for related Lewis antigens (e.g. sialyl Lewis x) that are involved in similar processes in other organs and have also been shown to substitute for CA19–9 in individuals lacking this glycan (49).

Patients with pancreatitis have an elevated risk of 2.7 to 16.5-fold for developing PDAC while individuals with hereditary pancreatitis have a 40–55% lifetime risk of developing pancreatic cancer (1, 5). Therapeutic options for pancreatitis patients are currently focused on treating the symptoms and little can be done to facilitate the resolution of idiopathic pancreatitis or to prevent its recurrence, highlighting the pressing need for new treatments. Prophylactic intervention could also be beneficial in the setting of recurrent or hereditary pancreatitis, and following certain routine procedures for which pancreatitis is a common outcome. For example, 3.5% (1–16% range) of the >700,000 patients undergoing endoscopic retrograde cholangiopancreatography (ERCP) each year in the United States will develop pancreatitis (50, 51) and several risk factors can be used to identify patients with elevated risk for ERCP-associated pancreatitis (40%) (52). Fully human CA19–9 antibodies have passed phase 1A clinical trials PET-imaging of pancreatic cancer (53), facilitating rapid translation of CA19–9 targeted therapy to the clinic for the treatment of pancreatitis patients. Therefore, not only would a new treatment strategy for pancreatitis itself serve an unmet need, there is also an intriguing possibility that effective pancreatitis treatment could also lead to prevention approaches for PDAC. Furthermore, such an approach may also reduce the severity of PDAC due to the fibroinflammatory and EGFR-activating properties of CA19–9 modified proteins, including Fibulin 3.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

We thank P.W. Maffuid and J. S. Lewis for the 5B1 antibodies. We acknowledge the assistance of the Cold Spring Harbor Laboratory Animal and Genetic Engineering, Animal and Tissue Imaging, Microscopy, Flow Cytometry, Antibody and Phage Display, and Mass Spectrometry Shared Resources; the New York University Experimental Pathology Immunohistochemistry Core Laboratory and Rodent Genetic Engineering Core; the Center for Comparative Medicine and Pathology at Memorial Sloan Kettering Cancer Center; and the University of Cincinnati Mouse Metabolic Phenotyping Center. The TROMA III (CK19) antibody was obtained from the Developmental Studies Hybridoma Bank, created by the NICHD of the NIH and maintained at the University of Iowa.

Funding. This work was supported by the Lustgarten Foundation and Pancreatic Cancer UK. DAT is also supported by the Cold Spring Harbor Laboratory Association, the Cold Spring Harbor Laboratory and Northwell Health Affiliation, and the National Institutes of Health (NIH) 5P30CA45508–29, 5P50CA101955–07, P20CA192996–03, 1U10CA180944–04, 1R01CA188134–01, and 1R01CA190092–04 for DAT; U01CA210240–01A1 for DAT and MAH; P01CA217798 and P50CA127297 for MAH 5T32CA148056 and 5K99CA204725 for DDE; and R50CA211506 for YP.

In addition, we are grateful for the following support - SWOG ITSC 5U10CA180944–04 (DAT, HT). The CSHL Shared Resources are funded by the NIH Cancer Center Support Grant 5P30CA045508. The NYU Experimental Pathology Immunohistochemistry Core Laboratory and Rodent Genetic Engineering Core are supported in part by the Laura and Isaac Perlmutter Cancer Center Support Grant; NIH/NCI P30CA016087 and the National Institutes of Health S10 Grants; NIH/ORIP S10OD01058 and S10OD018338.

References and Notes

1. Yadav D, Lowenfels AB, The epidemiology of pancreatitis and pancreatic cancer. *Gastroenterology* 144, 1252–1261 (2013). [PubMed: 23622135]
2. Forsmark CE, Vege SS, Wilcox CM, Acute Pancreatitis. *N Engl J Med* 375, 1972–1981 (2016). [PubMed: 27959604]
3. Frossard J-L, Steer ML, Pastor CM, Acute pancreatitis. *The Lancet* 371, 143–152 (2008).
4. van Brummelen SE, Venneman NG, van Erpecum KJ, VanBerge-Henegouwen GP, Acute idiopathic pancreatitis: does it really exist or is it a myth? *Scandinavian journal of gastroenterology. Supplement*, 117–122 (2003).
5. Lowenfels AB et al., Pancreatitis and the risk of pancreatic cancer. International Pancreatitis Study Group. *N Engl J Med* 328, 1433–1437 (1993). [PubMed: 8479461]
6. Guerra C et al., Chronic pancreatitis is essential for induction of pancreatic ductal adenocarcinoma by K-Ras oncogenes in adult mice. *Cancer Cell* 11, 291–302 (2007). [PubMed: 17349585]
7. Zhang Y et al., Tumor markers CA19–9, CA242 and CEA in the diagnosis of pancreatic cancer: a meta-analysis. *Int J Clin Exp Med* 8, 11683–11691 (2015). [PubMed: 26380005]
8. Haglund C, Lindgren J, Roberts PJ, Nordling S, Gastrointestinal cancer-associated antigen CA 19–9 in histological specimens of pancreatic tumours and pancreatitis. *Br J Cancer* 53, 189–195 (1986). [PubMed: 3513813]
9. Makovitzky J, The distribution and localization of the monoclonal antibody-defined antigen 19–9 (CA19–9) in chronic pancreatitis and pancreatic carcinoma. An immunohistochemical study. *Virchows Archiv. B, Cell pathology including molecular pathology* 51, 535–544 (1986). [PubMed: 2878526]
10. Itzkowitz SH et al., Immunohistochemical comparison of Lea, monosialosyl Lea (CA 19–9), and disialosyl Lea antigens in human colorectal and pancreatic tissues. *Cancer research* 48, 3834–3842 (1988). [PubMed: 3288336]
11. Iwase K et al., Immunohistochemical study of neuron-specific enolase and CA 19–9 in pancreatic disorders. The value of neuron-specific enolase as a marker for islet cell and nerve tissue. *Gastroenterology* 91, 576–580 (1986). [PubMed: 3015709]

12. Malesci A et al., Determination of CA 19–9 antigen in serum and pancreatic juice for differential diagnosis of pancreatic adenocarcinoma from chronic pancreatitis. *Gastroenterology* 92, 60–67 (1987). [PubMed: 3465666]
13. Safi F et al., High sensitivity and specificity of CA 19–9 for pancreatic carcinoma in comparison to chronic pancreatitis. Serological and immunohistochemical findings. *Pancreas* 2, 398–403 (1987). [PubMed: 3306667]
14. Steinberg WM et al., Comparison of the sensitivity and specificity of the CA19–9 and carcinoembryonic antigen assays in detecting cancer of the pancreas. *Gastroenterology* 90, 343–349 (1986). [PubMed: 2416628]
15. Pinho SS, Reis CA, Glycosylation in cancer: mechanisms and clinical implications. *Nat Rev Cancer* 15, 540–555 (2015). [PubMed: 26289314]
16. Thomsen M et al., Prognostic role of carcinoembryonic antigen and carbohydrate antigen 19–9 in metastatic colorectal cancer: a BRAF-mutant subset with high CA 19–9 level and poor outcome. *Br J Cancer* 118, 1609–1616 (2018). [PubMed: 29872151]
17. Shi C, Merchant N, Newsome G, Goldenberg DM, Gold DV, Differentiation of pancreatic ductal adenocarcinoma from chronic pancreatitis by PAM4 immunohistochemistry. *Archives of pathology & laboratory medicine* 138, 220–228 (2014). [PubMed: 24476519]
18. Xia B, Feasley CL, Sachdev GP, Smith DF, Cummings RD, Glycan reductive isotope labeling for quantitative glycomics. *Anal Biochem* 387, 162–170 (2009). [PubMed: 19454239]
19. Narimatsu H, in *Handbook of Glycosyltransferases and Related Genes*, Taniguchi Net al., Eds. (Springer Japan, 2002), pp. 218–225.
20. Ishijima N et al., BabA-mediated adherence is a potentiator of the *Helicobacter pylori* type IV secretion system activity. *J Biol Chem* 286, 25256–25264 (2011). [PubMed: 21596743]
21. Sperandio M, Gleissner CA, Ley K, Glycosylation in immune cell trafficking. *Immunological reviews* 230, 97–113 (2009). [PubMed: 19594631]
22. Yue T et al., Enhanced discrimination of malignant from benign pancreatic disease by measuring the CA 19–9 antigen on specific protein carriers. *PLoS One* 6, e29180 (2011). [PubMed: 22220206]
23. Yue T et al., Identification of blood-protein carriers of the CA 19–9 antigen and characterization of prevalence in pancreatic diseases. *Proteomics* 11, 3665–3674 (2011). [PubMed: 21751362]
24. Hirao Y, Ogasawara S, Togayachi A, Identification of Core Proteins Carrying the Sialyl Lewis a Epitope in Pancreatic Cancers Identification of Core Proteins Carrying the Sialyl Lewis a Epitope in Pancreatic Cancers. *Journal of Molecular Biomarkers & Diagnosis* 03, (2012).
25. Dow LE et al., Conditional reverse tet-transactivator mouse strains for the efficient induction of TRE-regulated transgenes in mice. *PLoS One* 9, e95236 (2014). [PubMed: 24743474]
26. Beard C, Hochedlinger K, Plath K, Wutz A, Jaenisch R, Efficient method to generate single-copy transgenic mice by site-specific integration in embryonic stem cells. *Genesis* 44, 23–28 (2006). [PubMed: 16400644]
27. Whitcomb DC et al., Chronic pancreatitis: An international draft consensus proposal for a new mechanistic definition. *Pancreatology* 16, 218–224 (2016). [PubMed: 26924663]
28. Takada A et al., Adhesion of human cancer cells to vascular endothelium mediated by a carbohydrate antigen, sialyl Lewis A. *Biochem Biophys Res Commun* 179, 713–719 (1991). [PubMed: 1716885]
29. Takada A et al., Contribution of carbohydrate antigens sialyl Lewis A and sialyl Lewis X to adhesion of human cancer cells to vascular endothelium. *Cancer research* 53, 354–361 (1993). [PubMed: 7678075]
30. Majumder S, Chari ST, Chronic pancreatitis. *The Lancet* 387, 1957–1966 (2016).
31. Struyvenberg MR, Martin CR, Freedman SD, Practical guide to exocrine pancreatic insufficiency - Breaking the myths. *BMC Med* 15, 29 (2017). [PubMed: 28183317]
32. Falk PG, Bry L, Holgersson J, Gordon JI, Expression of a human alpha-1,3/4-fucosyltransferase in the pit cell lineage of FVB/N mouse stomach results in production of Leb-containing glycoconjugates: a potential transgenic mouse model for studying *Helicobacter pylori* infection. *Proc Natl Acad Sci U S A* 92, 1515–1519 (1995). [PubMed: 7878011]

33. Habtezion A, Inflammation in acute and chronic pancreatitis. *Current opinion in gastroenterology* 31, 395–399 (2015). [PubMed: 26107390]
34. Inman KS, Francis AA, Murray NR, Complex role for the immune system in initiation and progression of pancreatic cancer. *World journal of gastroenterology* 20, 11160–11181 (2014). [PubMed: 25170202]
35. Klöppel G, Adsay NV, Chronic Pancreatitis and the Differential Diagnosis Versus Pancreatic Cancer. *Archives of pathology & laboratory medicine* 133, 382–387 (2009). [PubMed: 19260744]
36. Ardito CM et al., EGF receptor is required for KRAS-induced pancreatic tumorigenesis. *Cancer Cell* 22, 304–317 (2012). [PubMed: 22975374]
37. Navas C et al., EGF receptor signaling is essential for k-ras oncogene-driven pancreatic ductal adenocarcinoma. *Cancer Cell* 22, 318–330 (2012). [PubMed: 22975375]
38. Boj Sylvia F. et al., Organoid Models of Human and Mouse Ductal Pancreatic Cancer. *Cell* 160, 324–338 (2015). [PubMed: 25557080]
39. Tiriach H et al., Organoid profiling identifies common responders to chemotherapy in pancreatic cancer. *Cancer Discov*, (2018).
40. Vieira AV, Lamaze C, Schmid SL, Control of EGF receptor signaling by clathrin-mediated endocytosis. *Science* 274, 2086–2089 (1996). [PubMed: 8953040]
41. Bill HM et al., Epidermal growth factor receptor-dependent regulation of integrin-mediated signaling and cell cycle entry in epithelial cells. *Mol Cell Biol* 24, 8586–8599 (2004). [PubMed: 15367678]
42. Wang F et al., Reciprocal interactions between beta1-integrin and epidermal growth factor receptor in three-dimensional basement membrane breast cultures: a different perspective in epithelial biology. *Proc Natl Acad Sci U S A* 95, 14821–14826 (1998). [PubMed: 9843973]
43. Liu YC et al., Sialylation and fucosylation of epidermal growth factor receptor suppress its dimerization and activation in lung cancer cells. *Proc Natl Acad Sci U S A* 108, 11332–11337 (2011). [PubMed: 21709263]
44. Matsumoto K et al., N-Glycan fucosylation of epidermal growth factor receptor modulates receptor activity and sensitivity to epidermal growth factor receptor tyrosine kinase inhibitor. *Cancer science* 99, 1611–1617 (2008). [PubMed: 18754874]
45. Camaj P et al., EFEMP1 binds the EGF receptor and activates MAPK and Akt pathways in pancreatic carcinoma cells. *Biol Chem* 390, 1293–1302 (2009). [PubMed: 19804359]
46. Sandgren EP, Luetke NC, Palmiter RD, Brinster RL, Lee DC, Overexpression of TGF alpha in transgenic mice: induction of epithelial hyperplasia, pancreatic metaplasia, and carcinoma of the breast. *Cell* 61, 1121–1135 (1990). [PubMed: 1693546]
47. Jackson EL et al., Analysis of lung tumor initiation and progression using conditional expression of oncogenic K-ras. *Genes Dev* 15, 3243–3248 (2001). [PubMed: 11751630]
48. Hingorani SR et al., Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. *Cancer Cell* 4, 437–450 (2003). [PubMed: 14706336]
49. Tang H et al., Glycan motif profiling reveals plasma sialyl-lewis x elevations in pancreatic cancers that are negative for sialyl-lewis A. *Mol Cell Proteomics* 14, 1323–1333 (2015). [PubMed: 25733690]
50. Elmunzer BJ, Prevention of ERCP-induced pancreatitis. *Pancreapedia: The Exocrine Pancreas Knowledge Base*, (2015).
51. Parekh PJ, Majithia R, Sikka SK, Baron TH, The “Scope” of Post-ERCP Pancreatitis. *Mayo Clin Proc* 92, 434–448 (2017). [PubMed: 28160947]
52. Freeman ML et al., Complications of Endoscopic Biliary Sphincterotomy. *New England Journal of Medicine* 335, 909–919 (1996). [PubMed: 8782497]
53. Sawada R et al., Human monoclonal antibodies to sialyl-Lewis (CA19.9) with potent CDC, ADCC, and antitumor activity. *Clin Cancer Res* 17, 1024–1032 (2011). [PubMed: 21343375]
54. Vizcaino JA et al., 2016 update of the PRIDE database and its related tools. *Nucleic Acids Res* 44, 11033 (2016). [PubMed: 27683222]
55. Edgar R, Domrachev AE, Lash M, Lash AE, Gene Expression Omnibus: NCBI gene expression and hybridization array data repository.

Fig. 1. *FUT3* with $\beta 3GALT5$ expression enables CA19–9 production in engineered mouse pancreatic cancer cells.

(A) Ectopic *FUT3* induces Lewis^x (Le^x) but not CA19–9/sialyl-Lewis^a (sLe^a) expression in mouse PDAC cells by flow cytometric analysis. The Le^x and CA19–9/sLe^a positive human cell line Colo205 and Le^x and CA19–9/sLe^a negative parental KPC cell lines are shown. (B) CA19–9 flow cytometry of mouse PDAC cells stably and constitutively expressing *FUT3* with $\beta 3GALT5$ (“FB”) compared to the isotype control antibody. (C) Overlap between CA19–9 protein carriers identified in 3 out of 3 human PDAC cell lines (n = 926) with three independent mouse PDAC cell lines expressing *FUT3* and $\beta 3GALT5$.

Fig. 2. CA19-9 expression promotes pancreatitis in mice.

(A) Histologic evaluation of C;R^{LSL};F mice by hematoxylin and eosin staining (H&E), Masson's trichrome staining (MT, blue indicates collagen deposition) and CA19-9 expression (open arrow, CA19-9⁺ duct; closed arrow, CA19-9⁺ islet) by IHC following treatment with Dox. Scale bars = 50μm. (B) Quantification of the percentage pancreatic area exhibiting histologic signs of pancreatitis following treatment of C;R^{LSL};F mice with Dox. (C) Circulating levels (U/L) of the pancreatic enzymes amylase and lipase in C;R^{LSL};F mice following treatment with Dox (Days). The dotted line indicates the threshold elevation required for the diagnosis of pancreatitis. The dashed line indicates the maximum level of detection possible for amylase. (D) The circulating level of CA19-9 (U/ml) following treatment of C;R^{LSL};F mice with Dox. Values that exceed 37U/ml (dotted line) are elevated. (E) Immune cell infiltration evaluated by flow cytometry in mice treated with PBS or Cerulein for 2 days followed by a 1 or 3 day recovery period (C57Bl/6j, n = 5, 5, and 5, respectively) and C;R^{LSL};F mice treated with Dox (n = 4, 4, and 6, respectively) compared to "genetically negative" controls (GN, n = 3 and 3, respectively). Outlier analysis using Grubb's method identified one data point (triangle symbol, GN, 3 days of Dox) as an outlier. (F) EdU incorporation in the pancreas was evaluated by flow cytometry in C;R^{LSL};F mice (n

= 3, 3, and 4, respectively) and genetically negative littermate controls (GN, n = 3 and 2, respectively) following treatment with Dox.

Middle horizontal red lines represent the mean and error bars represent the standard deviation; each data point represents a measurement from an individual mouse. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ for multiple comparisons using Holm-Sidak's procedure following a one-way ANOVA.

Fig. 3. CA19-9 expression activates EGFR signaling.

(A) Representative phosphorylated-EGFR IHC in C;R^{LSL};F mice that were treated for 0 (n = 4) or 3 (n = 5) days with Dox. Insets are higher magnification of pancreatic ducts. Scale bars = 50µm. (B) GSEA of C;R^{LSL};F organoids identified enrichment in PI3K/AKT/MTOR and ECM Receptor signaling and downstream effector pathways. (C) C;R^{LSL};F organoids (n = 3 biological replicates) and genetically negative organoids (GN, n = 2 biological replicates) were evaluated by immunoblot for the activation of signaling pathways following treatment with Dox. Blots are representative of three technical replicates, three biological replicates of C;R^{LSL};F organoids and two biological replicates of GN organoids. (D) Quantification of changes to the ratio of phosphorylated to total protein is shown for Figures 3E, 3F, and 3G. (E-F) Organoids from GN littermates were incubated with conditioned media from C;R^{LSL};F organoids previously treated with Dox (24 hours) in the presence of Fc-control or Egfr-conjugated Fc and evaluated by immunoblot. (G) C;R^{LSL};F organoids (n = 3 biological replicates) were treated with Dox (Hours) in the presence of isotype control (MOPC21) or CA19-9 blocking mAb (NS19-9) and evaluated by immunoblot. Blots are representative of two technical and three biological replicates.

Figure 4. CA19-9 modified Fibulin 3 activates Egfr.

(A) IP/MS of EGFR complexes from C;R^{LSL};F (n=3) and GN organoid (n=2) whole cell lysates following Dox administration. (B) CA19-9 protein carrier IP/MS analysis of Fibulin 3 from conditioned media from C;R^{LSL};F organoids treated with Dox for 0 – 8 hours (n = 3) relative to untreated and treated GN organoids (n = 1) using 5B1 CA19-9 antibody clone. (C) Immunoblot of immunoprecipitated Fibulin 3 (Flag) following incubation with lysates from pancreatic cancer cells lacking Flag-Fbln3 expression. (D) C;R^{LSL};F organoids (n = 3) treated with Dox in the presence of MOPC21 or three independent Fibulin 3 antibodies and evaluated by immunoblot. Changes to the ratio of phosphorylated to total protein levels are included. (E) Organoids transduced with hairpins to GFP or to Fbln3 were immunoblotted following Dox treatment. Changes to the ratio of phosphorylated to total protein levels are included.

Middle horizontal red lines represent the mean and error bars represent the standard deviation; each data point represents a biological replicate. * p<0.05 by unpaired, two-tailed t test.

Figure 5. CA19-9 as a therapeutic target for the treatment of pancreatitis.

(A) H&E staining of representative areas from C;R^{LSL};F mice following treatment with human Isotype control (hIgG1, n = 5) or the CA19-9 antibody clone 5B1 (n = 5) for 8 days and Dox for the last 7 days. Scale bars = 100 μ m. (B) Serum amylase and lipase levels, and in C;R^{LSL};F mice treated with 5B1 or isotype control. (C) treated with either Isotype or 5B1 as described in Fig. 5A. Scale bars = 50 μ m.

Middle horizontal red lines represent the mean and error bars represent the standard deviation; each data point represents a measurement from an individual mouse. p value determined using unpaired, parametric, two-tailed t test. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

Figure 6. CA19-9 promotes rapid and aggressive pancreatic tumorigenesis.

(A) Survival curve for untreated (8 deaths out of 19 mice) and Dox treated K;C;R^{LSL}:F mice (14 deaths out of 19 mice) and KC genetic negative controls (13 deaths out of 34 mice). p value determined by Log-rank Mantel-Cox test. (B) Representative histology of pancreatic tumors and metastatic lesions from Dox treated K;C;R^{LSL}:F mice. Scale bars = 200μm. (C) Representative histology (scale bars = 100μm). (D) CA19-9 IHC (scale bars = 100μm), and (E) phospho-EGFR IHC (scale bars = 50μm) of the pancreata from K;C and K;C;R^{LSL}:F mice following 2 – 4 weeks of Dox treatment.