Activin A Modulates Inflammation in Acute Pancreatitis and Strongly Predicts Severe Disease Independent of Body Mass Index

Alexandra L. Thomas, PhD[1], Karla Castellanos, MS[1], Georgina Mancinelli, BS[1], Yinglin Xia, PhD[1], Jessica Bauer, PhD[2], Cemal Yazici, MD, MS[1], Giamila Fantuzzi, PhD[3], Rosa F. Hwang, MD, FACS[4], Nancy L. Krett, PhD[1], Georgios I. Papachristou, MD, PhD[5], David C. Whitcomb, MD, PhD, FACG[6] and Barbara Jung, MD[1,7]

INTRODUCTION: **Acute pancreatitis (AP) is a healthcare challenge with considerable mortality. Treatment is limited to supportive care, highlighting the need to investigate disease drivers and prognostic markers. Activin A is an established mediator of inflammatory responses, and its serum levels correlate with AP severity. We hypothesized that activin A is independent of body mass index (BMI) and is a targetable promoter of the AP inflammatory response.**

METHODS: **We assessed whether BMI and serum activin A levels are independent markers to determine disease severity in a cohort of patients with AP. To evaluate activin A inhibition as a therapeutic, we used a cerulein-induced murine model of AP and treated mice with activin A-specific neutralizing antibody or immunoglobulin G control, both before and during the development of AP. We measured the production and release of activin A by pancreas and macrophage cell lines and observed the activation of macrophages after activin A treatment.**

RESULTS: **BMI and activin A independently predicted severe AP in patients. Inhibiting activin A in AP mice reduced disease severity and local immune cell infiltration. Inflammatory stimulation led to activin A production and release by pancreas cells but not by macrophages. Macrophages were activated by activin A, suggesting activin A might promote inflammation in the pancreas in response to injury.**

DISCUSSION: **Activin A provides a promising therapeutic target to interrupt the cycle of inflammation and tissue damage in AP progression. Moreover, assessing activin A and BMI in patients on hospital admission could provide important predictive measures for screening patients likely to develop severe disease.**

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/CTG/A284

Clinical and Translational Gastroenterology 2020;11:e00152. https://doi.org/10.14309/ctg.0000000000000152

INTRODUCTION

Acute pancreatitis (AP) is the rapid onset of inflammation of the pancreas in response to injury. Pancreatitis is one of the most common causes of gastrointestinal-related hospitalizations and a considerable healthcare burden (1,2). Although most of the cases will resolve with time, a subset of patients will develop organ failure with mortality rates reaching 20% in patients with severe AP (3–5). Currently, there is no validated model to predict AP severity. Furthermore, current treatment is restricted to supportive care (6,7).

The mechanisms underlying the development of AP and the progression to severe disease are unclear. Triggering events lead to an uncontrolled inflammatory response (2), and the intensity of inflammation is linked to disease severity (8,9). After injury, innate immune cells, primarily neutrophils and macrophages, rapidly infiltrate the pancreas (10,11), resulting in damaging levels of cytokines, termed a "cytokine storm," leading to tissue damage, organ failure, and impaired pancreas regeneration (9,12,13). However, although inhibition of major proinflammatory cytokines, such as tumor necrosis factor (TNF) and

[1]Gastroenterology and Hepatology, Department of Medicine, University of Illinois at Chicago, Chicago, Illinois, USA; [2]Department of Medicine, University of Washington College of Medicine, Seattle, Washington, USA; [3]Department of Kinesiology and Nutrition, University of Illinois at Chicago, Chicago, Illinois, USA; [4]Surgery, Department of Breast Surgical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA; [5]Gastroenterology, Hepatology and Nutrition, Department of Internal Medicine, The Ohio State University, Columbus, Ohio, USA; [6]Gastroenterology, Hepatology and Nutrition, Department of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, USA. [7]Current affiliation: Department of Medicine, University of Washington College of Medicine, Seattle, Washington, USA. **Correspondence:** Barbara Jung, MD. E-mail: bhjung@uw.edu.

Received October 2, 2019; accepted February 18, 2020; published online May 1, 2020

interleukin (IL) 6, has shown promise in preclinical models, it has not been translated to patient benefit (9).

Activin A, a member of the TGFβ superfamily, is a cytokine associated with the immune response (14–16) and plays a key role in various murine models of inflammatory disease, such as inflammatory bowel disease, asthma, viral infections, and obesity (16–21). We have previously shown that serum activin A levels in patients with AP positively correlate with disease severity and that inhibition of activin A in a murine model of severe AP drastically reduced the initiation of disease-related mortality (22).

In our previous assessment of serum activin A levels as a marker for AP disease severity (22), we did not consider the impact of body mass index (BMI), a known risk factor of patients developing severe disease (23,24). Serum activin A levels are increased in subjects with BMI more than 30 (25), leading to the concern that elevated serum activin A levels in severe AP might reflect BMI status. In this report, using the same clinical cohort, we examined whether elevated serum activin A levels in patients with AP are independent of BMI. Using the murine AP model of IL12/IL18 injection in *ob*/*ob* mice, we previously reported that blocking activin A at the initiation of AP was effective in reducing the severity of AP (22). In this study, we verify that observation using the well-established cerulein injection murine model. We provide additional studies to address the therapeutic potential of blocking activin A once AP is established. In addition, we examine the role of activin A on the immune response during the development of AP.

METHODS

Cell lines, culture, and stimulants

Human pancreas stellate cells (hPSC) and human ductal epithelial cells (HPDE) were a generous gift from Dr Paul Grippo (University of Illinois at Chicago [UIC]). HPSC cells were originally developed by Dr Rosa Hwang (The University of Texas MD Anderson Cancer Center) (26). RAW264.7 cells were a generous gift from Dr Jun Sun (UIC). Cells were validated with CellCheck 9 Plus and tested for mycoplasma (IDEXX). HPSCs were maintained in DMEM supplemented with antibiotics and 10% fetal bovine serum (Invitrogen). HPDE cells were maintained in keratinocyte media supplemented with epidermal growth factor 1–53 and bovine pituitary extract (Gibco, Waltham, MA). RAW264.7 cells were maintained in Roswell Park Memorial Institution media (Gibco) supplemented with 10% fetal bovine serum. Cells were plated with serum-free media 24 hours before any treatments. To simulate an inflammatory environment, cells were stimulated with TNF (1–100 ng/mL) or Lipopolysaccaride (LPS) (0.1–10 μg/mL) (Cell Signaling Technology). RAW264.7 cells were treated with 25 ng/mL of activin A peptide (R&D Systems, Minneapolis, MN). Phosphate-buffered saline was used as a control treatment.

Murine models of AP

Female C57BL mice (Charles River Laboratories), aged 9–10 weeks, received 12 hourly intraperitoneal injections of cerulein at 50 μg/kg (Sigma-Aldrich, Burlington, MA). Control mice received equal volume of phosphate-buffered saline injections. To inhibit activin A, mice received a single injection of activin A-specific mouse monoclonal neutralizing antibody (AF388; R&D Systems) at 2 mg/kg either 30 minutes before the start of cerulein injections or at 7 hours of cerulein injections. A monoclonal mouse IgG_1 isotype (R&D Systems) injection of equal volume and concentration was given as a control treatment. All animal protocols and experiments were approved by the Animal Care and Use Committee of UIC and performed in accordance with their guidelines and regulations.

Murine sample preparation and assessment

Blood was mixed with heparin and centrifuged to isolate plasma. Pancreatic tissue was fixed in 10% formalin, paraffin embedded, and sectioned as previously described (22). Tissue sections were stained with hematoxylin and eosin (H&E) and scored for edema, necrosis, disease severity, and neutrophil infiltration as previously described (22). Immunohistochemical staining of CD68 (ab955; Abcam), TGFβ1 (ab125287; Abcam), and inhibin βA (subunit of activin A) (AI006; Ansh Labs, Webster, TX) was performed as previously described (27). In all cases, slides were blindly scored by 2 independent investigators and received scores of 0 (no staining), 1 (minimal staining), 2 (moderate staining), or 3 (high staining).

Protein and mRNA detection

ELISA. Activin A from murine plasma and cell media supernatant was measured with the activin A Quantikine ELISA kit (R&D Systems, Minneapolis, MN) following the manufacturer's instructions. Plasma samples and hPSC supernatants were diluted 1:2 and 1:3, respectively. α-Amylase was measured using a kit from Teco Diagnostics (Anaheim, CA) according to the manufacturer's instructions. Samples were assayed in duplicate.

Immunoblotting and antibodies. Immunoblotting was performed as previously described (28) with the following antibodies: mouse monoclonal to inhibin βA (AI006; Ansh), mouse monoclonal to α-tubulin (3873; Cell Signaling Technology), and rabbit polyclonal to β-actin (4967; Cell Signaling Technology).

Quantitative real-time polymerase chain reaction. Total RNA was isolated from study cells with the RNeasy Plus Kit (Qiagen, Waltham, MA) following manufacturer's instructions. Complementary DNA was synthesized with the SuperScript Vilo cDNA Synthesis Kit (Invitrogen, Waltham, MA). TaqMan gene expression assays for *INHBA*, *TNF*, and *IL1B* were used with TaqMan Fast Advanced Master Mix according to manufacturer's instructions (Applied Biosystems, Waltham, MA). Quantitative real-time polymerase chain reaction was performed on a ViiaA7 instrument (Applied Biosystems). The results were normalized to 2 independent control genes (*RPL13A* and *B2M* [human] or *MRPL19* [mouse]) using GeNorm software (29).

Human cohort with pancreatitis

A detailed description of this cohort has been previously published (22). Subjects were recruited at the University of Pittsburgh Medical Center, and all protocols were approved by the Institutional Review Board of University of Pittsburgh. An informed consent was obtained for all participants. Deidentified patient serum samples (from 30 control subjects and from 10 patients with mild, 10 with moderate, and 10 with severe AP according to revised Atlanta criteria (3)) were analyzed at UIC, as previously described (22).

Statistical analysis

Data are presented as mean ± SD. All statistical tests were 2 sided, and P values <0.05 were considered statistically significant. In 1- or 2-sample cases, a paired or unpaired t test was performed as appropriate to determine significant differences between treatments and conditions. For continuous variables, 1-way analysis of variance or nonparametric Kruskal–Wallis tests were

conducted as appropriate; for categorical variables, χ^2 or Fisher exact tests were conducted as appropriate. The parametric or nonparametric *P* values were reported based on the normality test of distribution. Multivariate analyses were conducted using generalized estimating equations to model the disease severity by including activin A and BMI as predictors controlled by age and sex. Ordered logistic regression was performed to analyze disease severity by including disease severity as a response variable and 4 BMI/activin A groups and time as predictors. To test independence of activin A and BMI as predictors of disease severity, multiple collinearity analysis was conducted for disease severity, activin A, and BMI along with other covariates. Statistical analyses were conducted using SAS version 9.4 (SAS Institute) or Prism (GraphPad Software).

RESULTS

Serum activin A level and BMI are independent prognostic tools for patients with AP

It is difficult to predict which patients with AP will progress to severe disease. Previously, our group demonstrated a positive correlation between serum activin A levels of patients and disease severity (22), but we did not consider the impact of BMI in this analysis to determine whether elevated serum activin A levels are dependent or independent of BMI. Using that same cohort of patients (22), we reanalyzed these data using subject BMI as a variable. As described previously (22), blood samples from each patient were collected at admission, 24 hours postadmission, and between 48 and 72 hours postadmission.

The univariate generalized estimating equations modeling results show that both activin A (estimated $\beta = 1.43$, $P = 0.0002$) and BMI (estimated $\beta = 0.07$, $P = 0.0102$) positively predict the severe of disease individually with the different measurements of activin A having no effect on this prediction. The logistic regression also showed that activin A and BMI predict severe disease individually with odds ratios of comparing moderate or severe vs control or mild are 53.75 (95% confidence interval: 8.07–357.79), $P < 0.001$, for activin A, and 1.13 (95% confidence interval: 1.06–1.21), $P < 0.001$, for BMI, respectively. These results suggest that activin A and BMI were independent predictors of disease severity. The multiple collinearity test showed the nonexistence of collinearities among activin A, BMI, and other covariates (e.g., age and sex), which further indicates that activin A and BMI independently predict the disease severity.

High BMI is a known risk factor associated with severe AP (23,24), confirmed in a murine model of AP where lean mice expressed mild disease whereas obese mice exhibited sever disease (30). The BMI value of 30 has been widely accepted to indicate obese subjects (31), and therefore, we have used that value as our cutoff to indicate high BMI. The predictive value of BMI for severe AP is limited. As shown in Figure 1, there are patients with both mild and moderate AP with a BMI > 30 and patients with severe AP with a BMI ≤30. Of clinical relevance, there is no predictive measure for patients with low BMI who may progress to severe AP. Thus, we examined the serum activin A levels in the subgroup of patients with low BMI, defined as patients with BMI ≤30 (Figure 1b). In this group, activin A strongly predicted disease severity ($P < 0.01$). This suggests that patient's BMI and serum activin A levels can be used independently to predict the probability of patients developing severe AP and identify those that require more focused care.

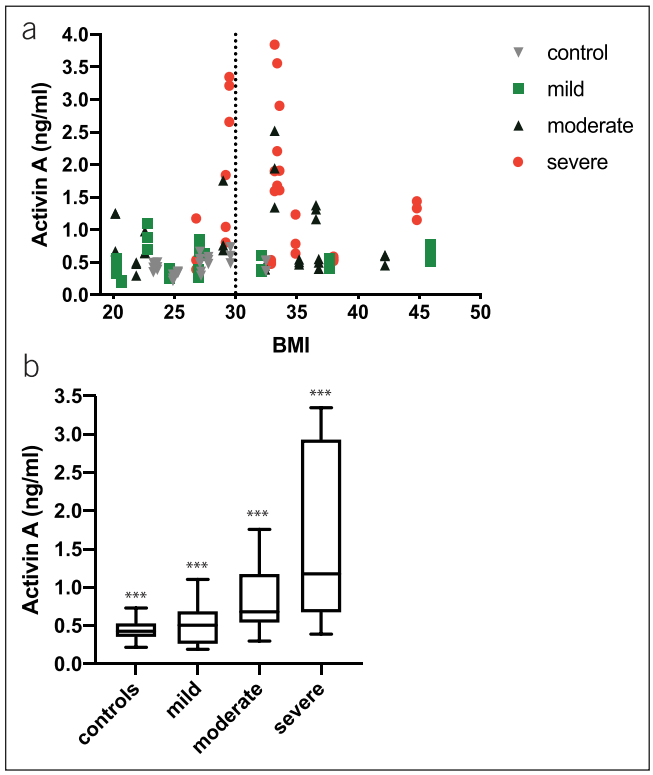

Figure 1. Serum activin A level of patient is independent of BMI as a prognostic tool for AP disease severity. (**a**) ELISA of serum activin A levels (y axis) plotted against the patient BMI (x axis) distributed by disease severity group (control = inverted triangle; mild AP = square; moderate AP = triangle; severe AP = circle) n = 10 for each group with activin A estimated at admission and 24 hr and 48–72 hr after admission for each patient. (**b**) Serum activin A levels in those subjects with a BMI ≤30. GEE multivariate analysis confirmed that serum activin A level is predictive of disease severity for patients with low BMI ($P < 0.0001$). AP, acute pancreatitis; BMI, body mass index; GEE, generalized estimating equation.

Activin A inhibition reduces inflammation and disease severity in AP murine model

In addition to being a powerful prognostic marker, previous data from our laboratory suggest that activin A has a mechanistic role in AP (22). To verify the requirement of activin A in the development of AP, we used a cerulein-induced murine model of AP. Thirty minutes before the 12 hourly cerulein injections (50 μg/kg), mice received either an activin A-specific neutralizing antibody (2 ng/kg) or an equal amount of immunoglobulin G (IgG) isotype control antibody. Mice were killed 24 hours after the first cerulein injection (Figure 2a). Amylase activity was measured to confirm the development of pancreatitis (see Figure 1A, Supplementary Digital Content 1, http://links.lww.com/CTG/A284), and activin A plasma levels were assessed by an activin A-specific ELISA. Treatment with the neutralizing antibody successfully reduced activin A to undetectable levels (see Figure 1B, Supplementary Digital Content 1, http://links.lww.com/CTG/A284).

H&E staining of mouse pancreatic tissue revealed significant differences in disease severity with activin A inhibition. Mice treated with the neutralizing antibody had less edema and necrosis compared with IgG control-treated mice (Figure 2b,C). Moreover, mice with activin A inhibition also had less inflammation. Neutrophil infiltration was significantly reduced, as was CD68 macrophage

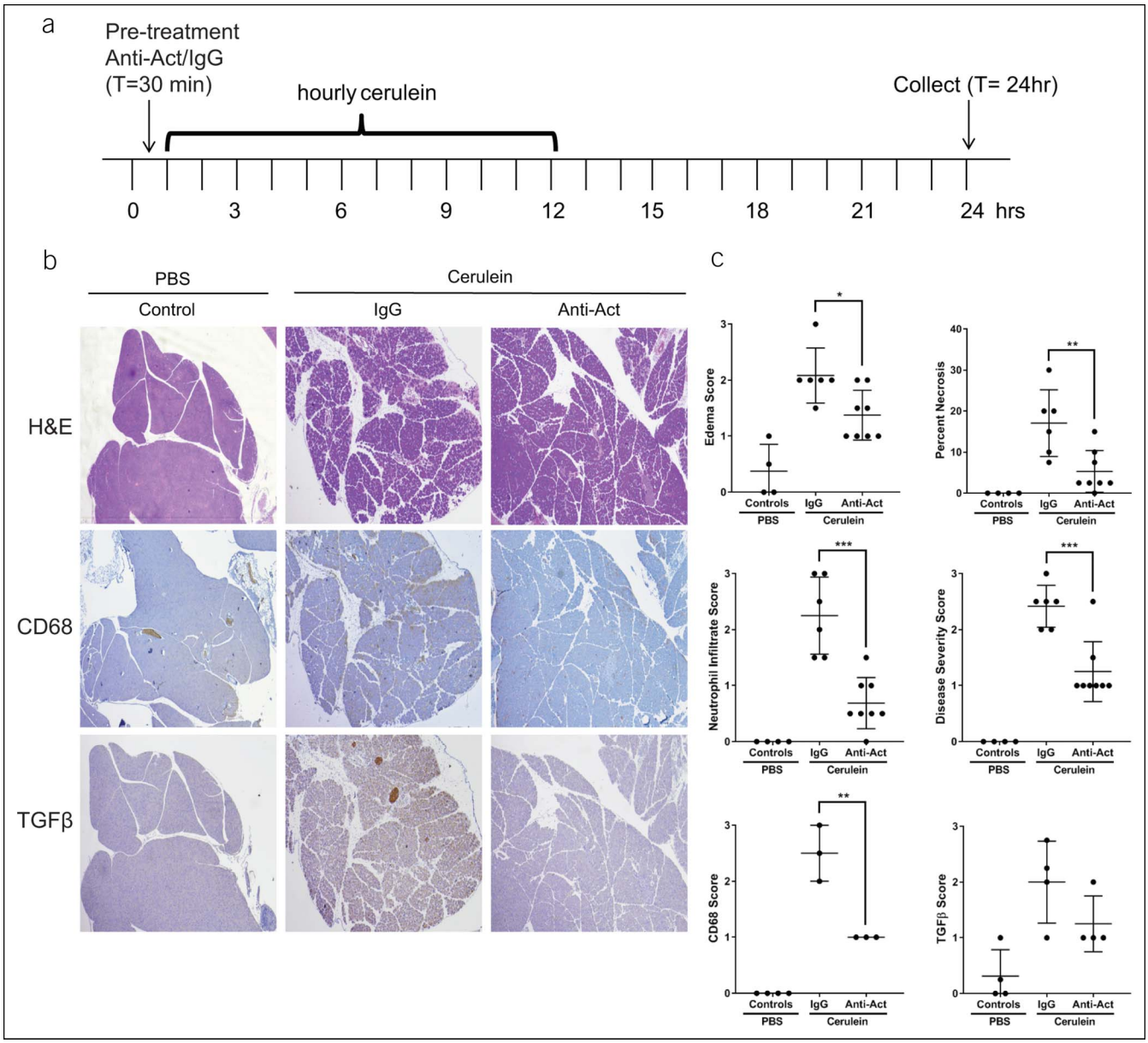

Figure 2. Inhibition of activin A in cerulein-induced AP murine model reduces disease severity and inflammation. (**a**) Schematic of treatment and cerulein injection schedule. Mice were pretreated with activin A-specific neutralizing antibody (Anti-Act) or IgG control 30 minutes before the start of cerulein injections. Mice were given 12 hourly injections of 50 μg/kg cerulein or equal volume PBS. Blood and tissue were collected 24 hr after the start of cerulein injections. (**b**) Stained sections of pancreatic tissue from mice with cerulein-induced AP. Top row, H&E staining; middle row, IHC staining of CD68; and bottom row, IHC staining of TGFβ. Representative images were taken at ×40 total magnification. (c) Quantification of pancreatic tissue staining. n = 5–10 mice per group. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. AP, acute pancreatitis; IHC, immunohistochemical; PBS, phosphate-buffered saline.

infiltration (Figure 2b,c). Overall, these data suggest that activin A is necessary for the full development of cerulein-induced pancreatitis and likely plays a role in immune cell infiltration of the pancreas.

TGFβ1 ligand, a relative of activin A ligand, increased in the pancreas with disease as expected (32), but did not change significantly with activin inhibition (Figure 2b,c), indicating the influence of activin A specifically on AP disease development. The anti-activin antibody was also administered at 7 hours of cerulein injection as an AP treatment (Figure 3a). Mice were collected at 24 hours to assess parameters of AP and at 7 days to assess regeneration. At 24 hours, the anti-activin treatment significantly reduced pancreatic edema (Figure 3b,c), necrosis (Figure 3b,d), pancreas:body weight ratio (Figure 3e), neutrophil infiltration (Figure 3b,f), and disease severity score (Figure 3g). The role of activin in tissue regeneration at the resolution of pancreatitis is controversial and requires additional studies. Reduction of pancreatic regeneration in patients with AP would be concerning. We analyzed the pancreata collected 7 days after initiation of AP and compared mice treated with a control IgG to those treated with anti-activin (Figure 3). By all the criteria measured, the anti-activin–treated pancreata returned to control values and appeared to have normal architecture on H&E staining. The

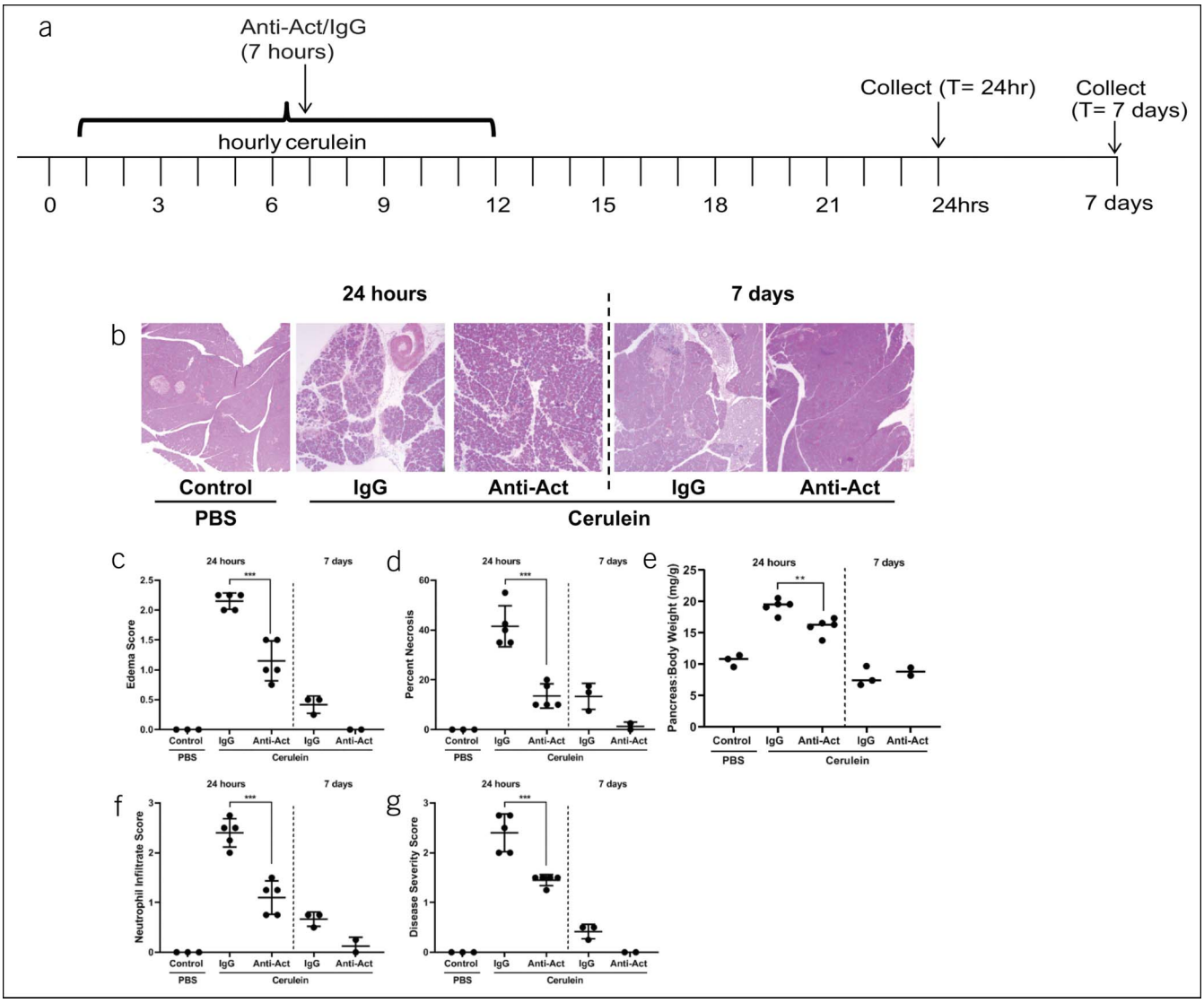

Figure 3. Inhibition of activin A treatment of cerulein-induced AP decreases disease severity but does not reduce pancreas regeneration. (**a**) Schematic of treatment and cerulein injection schedule. Mice (n = 3–5) were given 12 hourly injections of 50 μg/kg cerulein or equal volume PBS. At 7 hr of cerulein injection, mice were treated with activin A-specific neutralizing antibody (Anti-Act) or IgG control. Blood and tissue were collected 24 hr after the start of cerulein injections and at 7 d. (**b**) Hematoxylin/eosin stained pancreatic sections at ×10 magnification. (**c**) Edema score at 24 hr and 7 d of cerulein injection with and without Anti-Act. (**d**) Percentage of pancreatic necrosis, (**e**) pancreas:body weight ratio, (**f**) neutrophil infiltration score, and (**g**) AP severity score. $^{**}P < 0.01$, $^{***}P < 0.001$. AP, acute pancreatitis; PBS, phosphate-buffered saline.

IgG-treated mice had values above control for 4 of the measured assays indicating some residual disease.

Activin A is produced and released by pancreas cells but not macrophages

To delineate the relationship between activin A and an immune response during AP, we treated pancreatic hPSC and HPDE cell lines with TNF to simulate an inflammatory environment as seen in AP. Levels of *INHBA* mRNA, which encodes the βA subunit of activin A, increased significantly, indicating increased transcription of activin A precursors (Figure 4a). Accordingly, protein levels of the profrom βA subunit of activin A also increased in both cell lines (Figure 4b,c). βA subunits are processed and dimerize outside of the cell; thus, fully formed activin A cannot be detected by immunoblots of cell lysate (33).

Release of activin A from hPSC and HPDE cells was measured by activin A-specific ELISA of cell culture supernatants. Treatment with inducers of inflammatory responses, either TNF or LPS, caused an increase in activin A release in a dose-dependent manner (Figure 4d). Conversely, a macrophage cell line, RAW264.7, did not release detectable amounts of activin A in response to stimulation with TNF or LPS (Figure 4e), nor did TNF treatment induce an increase in *INHBA* mRNA production in these cells (Figure 4f). This suggests that activin A might come from the pancreas cells during AP.

Activin A activates macrophage cell lines

Despite not releasing activin A, macrophage cells were responsive to activin A treatment. RAW264.7 cells were treated with activin A peptide for 24 hours. Levels of *TNF* and *IL1B* mRNA more than

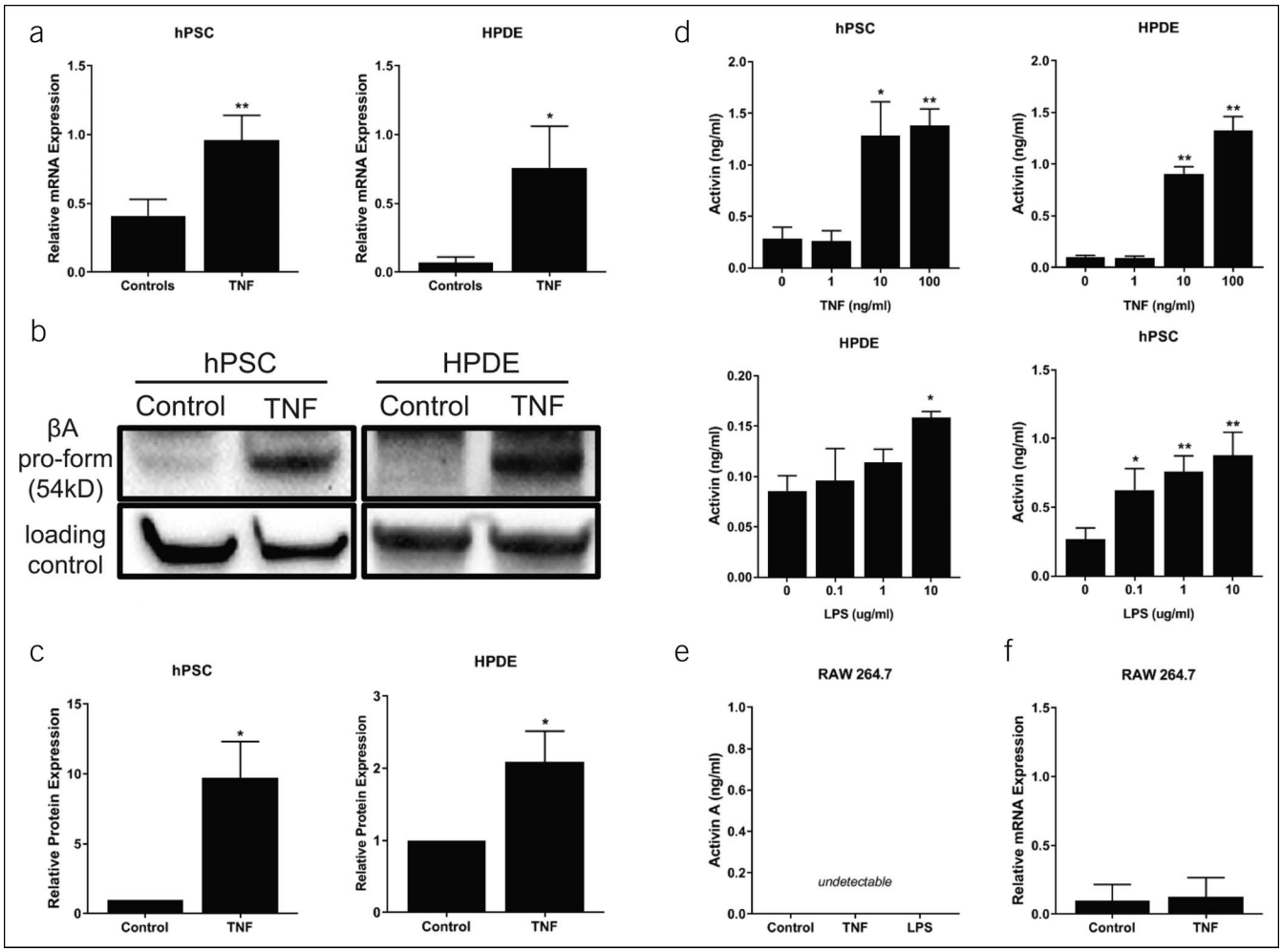

Figure 4. Activin A is produced and released by pancreatic cell lines but not by macrophage cell lines. (**a**) qRT-PCR results of *INHBA* mRNA levels in hPSC and HPDE cells after 24-hr treatment with 10 ng/mL TNF. (**b**) Representative immunoblots of the βA subunit proform of activin A in hPSC and HPDE cells after 24-hr treatment with 10 ng/mL TNF. Loading control is α-tubulin for hPSC cells and β-actin for HPDE cells. (**c**) Densitometry quantification of immunoblots of at least 3 independent experiments. (**d**) ELISA of activin A in cell culture media of hPSC and HPDE cells after treatment with increasing concentrations of TNF or LPS for 24 hr. (**f**) ELISA of activin A in cell culture media of RAW264.7 cells treated with 10 ng/mL TNF or 10 μg/mL LPS for 24 hr. Activin A in the media was undetectable. (**e**) *INHBA* mRNA level in RAW264.7 cells after activation with 10 ng/mL TNF. $*P < 0.05$; $**P < 0.01$. HPDE, human ductal epithelial cells; hPSC, human pancreas stellate cells; qRT-PCR, quantitative real-time polymerase chain reaction.

doubled with activin A treatment (Figure 5a,B), suggesting that macrophages can become activated in response to activin A. Taken together, our data suggest a model of AP where, after injury, stimulated pancreatic cells produce and release activin A that mediates an immune response, contributing to the inflammatory storm of AP that can lead to severe disease progression.

DISCUSSION

AP remains a critical public health challenge partly because of the inability to accurately predict patients who will develop severe disease, which can be lethal, and the lack of curative treatment options (3–7). In this study, we reexamined our clinical data and determined that elevated serum activin is independent of an elevated BMI as a marker of severe AP. This suggests serum activin A levels can be useful to predict the development of severe AP in patients with BMI ≤30. The data obtained from the cohort used in this study strongly support the need for a larger prospective study of activin A as a prognostic marker in AP. AP is a complex disease, and the limited sample size in this study prevented subgroup analysis.

In this study, we validated the necessity of activin A for the full development of AP in the cerulein model of pancreatitis. Mice pretreated with activin A-specific neutralizing antibody had significantly less severe disease than control mice. Thus, an immediate clinical application of activin A inhibition could be considered as a prophylactic before endoscopic retrograde cholangiopancreatography to prevent procedure-induced pancreatitis, which occurs in 3%–5% of cases (34). Additional studies will help to identify the appropriate timing of activin inhibition to inform clinical application.

In addition, we found that treatment with the anti-activin antibody after the initiation of cerulein-induced AP was effective in reducing disease. Clinical inhibition of activin A is feasible, because the safety has been demonstrated in patients suffering from chemotherapy-induced anemia and ovarian cancer (35,36).

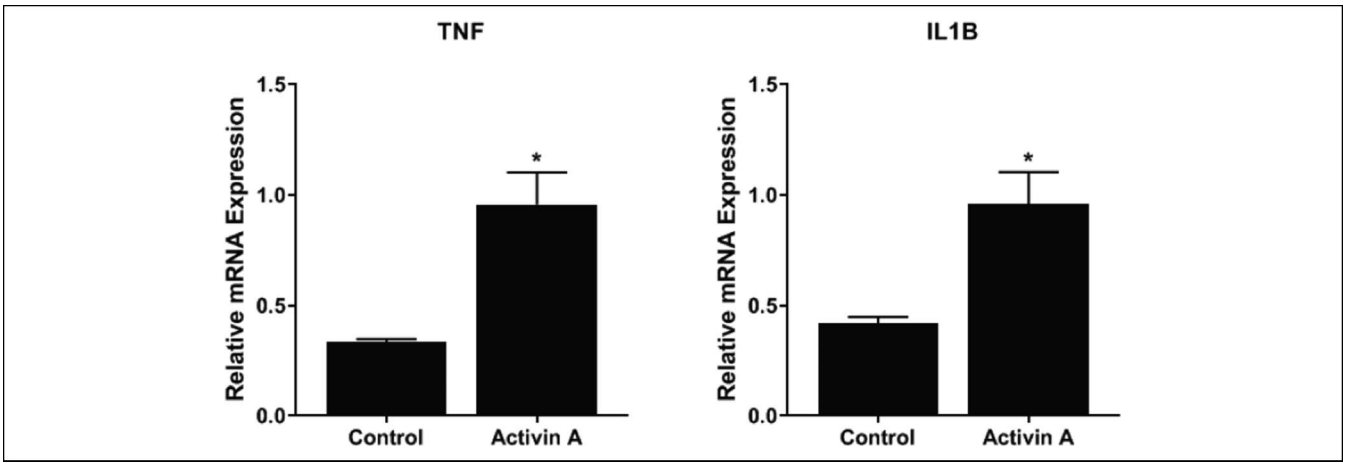

Figure 5. Activin A activates RAW264.7 macrophages. qRT-PCR results of *TNF* and *IL1B* mRNA expression in RAW264.7 macrophages after 24-hour treatment with 25 ng/mL activin A. $*P < 0.05$. qRT-PCR, quantitative real-time polymerase chain reaction.

Thus, with appropriate additional preclinical studies to examine timing of activin A peak levels, activin A inhibition could rapidly translate to clinical application. We have initiated a prospective clinical study to collect those data.

We further observed that the anti-activin treatment does not impair longer-term pancreas regeneration and might have assisted the regeneration process. One possibility is that anti-activin treatment prevented much of the cerulein-induced pancreatic damage and, therefore, the pancreata appear more normal at 7 days. It is also possible that inhibiting activin promotes repair of the pancreas. This would be useful in cases where patients present at the emergency department when their AP is fully developed and the therapeutic window to prevent an inflammatory response might be closed. However, if activin inhibition also promotes regeneration in addition to ameliorating the inflammatory response, there may be a broader clinical application for anti-activin therapy.

Using murine models of AP, we identified a link between activin A and innate immune cell infiltration of the pancreas. This closely correlated to disease severity. Our *in vitro* studies suggest that activin A is released by pancreatic cells and activates macrophages. This may be a key initiating element of the inflammatory response during AP that activin A inhibition could interrupt.

Dysregulation of cells of exocrine pancreas, primarily acinar and ductal epithelial cells, initiate pancreatitis, which, in turn, promotes an inflammatory response (37). Pancreatic stellate cells are understudied in AP, but it is known they can become activated in an inflammatory environment, such that occurs during AP. Stellate cells respond, in turn, by promoting fibrosis that can lead to chronic pancreatitis and the release of additional cytokines that prolong the immune response (38). In this report, we identified activin A as a potential signaling molecule released by pancreatic stellate and epithelial cells, which could act as a key factor in progressing the inflammatory response.

In conclusion, through *in vitro* models, murine models, and clinical data, we have demonstrated a key role of activin A in AP initiation and progression. Serum activin A level of patients acts a useful tool alone or in combination with patients' BMI to help predict patients in need for more focused care. Moreover, inhibition of activin A in patients is safe and promises to impede the deleterious inflammatory response that occurs during AP, which may ultimately improve patient outcomes.

CONFLICTS OF INTEREST

Guarantor of the article: Barbara Jung, MD.

Specific author contributions: A.L.T. designed study, collected, and interpreted data and composed figures and manuscript; K.C. assisted in study design and collected data; G.M. collected data; Y.X. performed statistical analysis; J.B. assisted in study design and figure preparation; C.Y. interpreted data; G.F. provided mouse tissue samples; R.F.H. developed human pancreatic stellate cells; N.L.K. assisted in study design, manuscript preparation, and final editing; G.P. and D.C.W. provided human cohort data; B.J. designed study, interpreted data, assisted in editing, and provided funding. All authors approved the submitted manuscript.

Financial Support: This project was supported by the National Center for Advancing Translational Science through grant no. UL1TR00203, which provided support for C.Y., and a pilot project grant to B.J. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Potential competing interests: None to report.

Study Highlights

WHAT IS KNOWN

- ✓ AP is a major healthcare burden.
- ✓ No curative treatments or reliable predictive measures currently exist.
- ✓ Activin A is a mediator of inflammation and is associated with severe AP.
- ✓ In phase I clinical trials, inhibition of activin A has a favorable safety profile.

WHAT IS NEW HERE

- ✓ Activin A is predictive of disease severity in patients independent of BMI. Activin A inhibition reduces inflammation and disease severity in mice with AP.
- ✓ Pancreatic cells are the likely source of activin A.

TRANSLATIONAL IMPACT

- ✓ Assessing activin A in patients with AP on hospital admission can be used to predict patients at risk for developing severe disease.

REFERENCES

1. Peery AF, Crockett SD, Murphy CC, et al. Burden and cost of gastrointestinal, liver, and pancreatic diseases in the United States: Update 2018. Gastroenterology 2019;156(1):254–72.e211.
2. Yadav D, Lowenfels AB. The epidemiology of pancreatitis and pancreatic cancer. Gastroenterology 2013;144(6):1252–61.
3. Banks PA, Bollen TL, Dervenis C, et al. Classification of acute pancreatitis—2012: Revision of the Atlanta Classification and Definitions by International Consensus. Gut 2013;62(1):102–11.
4. Beger HG, Rau BM. Severe acute pancreatitis: Clinical course and management. World J Gastroenterol 2007;13(38):5043–51.
5. Garg PK, Madan K, Pande GK, et al. Association of extent and infection of pancreatic necrosis with organ failure and death in acute necrotizing pancreatitis. Clin Gastroenterol Hepatol 2005;3(2):159–66.
6. Tenner S, Baillie J, DeWitt J, et al, American College of Gastroenterology. American College of Gastroenterology guideline: Management of acute pancreatitis. Am J Gastroenterol 2013;108(9):1400–15; 1416.
7. Vege SS, DiMagno MJ, Forsmark CE, et al. Initial medical treatment of acute pancreatitis: American Gastroenterological Association Institute Technical Review. Gastroenterology 2018;154(4):1103–39.
8. Gukovskaya AS, Gukovsky I, Algul H, et al. Autophagy, inflammation, and immune dysfunction in the pathogenesis of pancreatitis. Gastroenterology 2017;153(5):1212–26.
9. Gukovsky I, Li N, Todoric J, et al. Inflammation, autophagy, and obesity: Common features in the pathogenesis of pancreatitis and pancreatic cancer. Gastroenterology 2013;144(6):1199–209.e1194.
10. Habtezion A. Inflammation in acute and chronic pancreatitis. Curr Opin Gastroenterol 2015;31(5):395–9.
11. Montecucco F, Mach F, Lenglet S, et al. Treatment with evasin-3 abrogates neutrophil-mediated inflammation in mouse acute pancreatitis. Eur J Clin Invest 2014;44(10):940–50.
12. Folias AE, Penaranda C, Su AL, et al. Aberrant innate immune activation following tissue injury impairs pancreatic regeneration. PLoS One 2014; 9(7):e102125.
13. Makhija R, Kingsnorth AN. Cytokine storm in acute pancreatitis. J Hepatobiliary Pancreat Surg 2002;9(4):401–10.
14. Chen W, Ten Dijke P. Immunoregulation by members of the TGFbeta superfamily. Nat Rev Immunol 2016;16(12):723–40.
15. Jones KL, Mansell A, Patella S, et al. Activin A is a critical component of the inflammatory response, and its binding protein, follistatin, reduces mortality in endotoxemia. Proc Natl Acad Sci U S A 2007;104(41): 16239–44.
16. Morianos I, Papadopoulou G, Semitekolou M, et al. Activin-A in the regulation of immunity in health and disease. J Autoimmun 2019;104: 102314.
17. Hedger MP, Winnall WR, Phillips DJ, et al. The regulation and functions of activin and follistatin in inflammation and immunity. Vitam Horm 2011;85:255–97.
18. Koncarevic A, Kajimura S, Cornwall-Brady M, et al. A novel therapeutic approach to treating obesity through modulation of TGFbeta signaling. Endocrinology 2012;153(7):3133–46.
19. Linko R, Hedger MP, Pettila V, et al. Serum activin A and B, and follistatin in critically ill patients with influenza A(H1N1) infection. BMC Infect Dis 2014;14:253.
20. Zaragosi LE, Wdziekonski B, Villageois P, et al. Activin a plays a critical role in proliferation and differentiation of human adipose progenitors. Diabetes 2010;59(10):2513–21.
21. Zhang YQ, Resta S, Jung B, et al. Upregulation of activin signaling in experimental colitis. Am J Physiol Gastrointest Liver Physiol 2009;297(4): G768–780.
22. Staudacher JJ, Yazici C, Carroll T, et al. Activin in acute pancreatitis: Potential risk-stratifying marker and novel therapeutic target. Sci Rep 2017;7(1):12786.
23. Krishna SG, Hinton A, Oza V, et al. Morbid obesity is associated with adverse clinical outcomes in acute pancreatitis: A propensity-matched study. Am J Gastroenterol 2015;110(11):1608–19.
24. Martinez J, Johnson CD, Sanchez-Paya J, et al. Obesity is a definitive risk factor of severity and mortality in acute pancreatitis: An updated meta-analysis. Pancreatology 2006;6(3):206–9.
25. Zeller J, Kruger C, Lamounier-Zepter V, et al. The adipo-fibrokine activin A is associated with metabolic abnormalities and left ventricular diastolic dysfunction in obese patients. ESC Heart Fail 2019;6(2):362–70.
26. Hwang RF, Moore T, Arumugam T, et al. Cancer-associated stromal fibroblasts promote pancreatic tumor progression. Cancer Res 2008; 68(3):918–26.
27. Bauer J, Sporn JC, Cabral J, et al. Effects of activin and TGFbeta on p21 in colon cancer. PLoS One 2012;7(6):e39381.
28. Thomas AL, Lind H, Hong A, et al. Inhibition of CDK-mediated Smad3 phosphorylation reduces the Pin1-Smad3 interaction and aggressiveness of triple negative breast cancer cells. Cell Cycle 2017;16(15):1453–64.
29. Vandesompele J, De Preter K, Pattyn F, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 2002;3(7):RESEARCH0034.
30. Pini M, Sennello JA, Cabay RJ, et al. Effect of diet-induced obesity on acute pancreatitis induced by administration of interleukin-12 plus interleukin-18 in mice. Obesity (Silver Spring) 2010;18(3):476–81.
31. Purnell JQ. Definitions, classification, and epidemiology of obesity. In: Feingold KR, Anawalt B, Boyce A, et al (eds). Endotext. MDText.com.Inc: South Dartmouth, 2000.
32. Riesle E, Friess H, Zhao L, et al. Increased expression of transforming growth factor beta s after acute oedematous pancreatitis in rats suggests a role in pancreatic repair. Gut 1997;40(1):73–9.
33. Johnson KE, Makanji Y, Temple-Smith P, et al. Biological activity and in vivo half-life of pro-activin A in male rats. Mol Cell Endocrinol 2016;422: 84–92.
34. Parekh PJ, Majithia R, Sikka SK, et al. The "Scope" of post-ERCP pancreatitis. Mayo Clin Proc 2017;92(3):434–48.
35. Raftopoulos H, Laadem A, Hesketh PJ, et al. Sotatercept (ACE-011) for the treatment of chemotherapy-induced anemia in patients with metastatic breast cancer or advanced or metastatic solid tumors treated with platinum-based chemotherapeutic regimens: Results from two phase 2 studies. Support Care Cancer 2016;24(4):1517–25.
36. Tao JJ, Liu JF, Rasco DW, et al. Abstract CT011: First in human study of activin-A inhibitor, STM434, in patients with granulosa cell ovarian cancer and other advanced solid tumors. Cancer Res 2018;78(13 Supplement):CT011.
37. Habtezion A, Gukovskaya AS, Pandol SJ. Acute pancreatitis: A multifaceted set of organelle and cellular interactions. Gastroenterology 2019;156(7):1941–50.
38. Masamune A, Watanabe T, Kikuta K, et al. Roles of pancreatic stellate cells in pancreatic inflammation and fibrosis. Clin Gastroenterol Hepatol 2009;7(11 Suppl):S48–54.