AWARD NUMBER: W81XWH-21-1-0079

TITLE: Progenitor-Like Cells as an Etiological Factor and Potential Therapeutic Target in Alcohol-Induced Chronic Pancreatitis

PRINCIPAL INVESTIGATOR: JUN LIU

CONTRACTING ORGANIZATION: University of Texas Health Science Center, San Antonio, TX

REPORT DATE: October 2022

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
October 2022	Annual	01Sep2021-31Aug2022

4. TITLE AND SUBTITLE

Progenitor-Like Cells as an Etiological Factor and Potential Therapeutic Target in Alcohol-Induced Chronic Pancreatitis

5a. CONTRACT NUMBER
W81XWH-21-1-0079

5b. GRANT NUMBER
GRANT13112228

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
JUN LIU

E-Mail: LIUJ8@UTHSCSA.EDU

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Texas Health Science Center at San Antonio

7703 FLOYD CURL DR SAN ANTONIO TX 78229-3901

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

To examine the scientific hypothesis that disruption of ADM de-differentiation/ re-differentiation cycle induces hyper-sensitivity to chronic alcoholic pancreatitis and progenitor-like ADM cells can be targeted by the YAP1/TAZ pharmaceutical inhibitor Vivace102 to improve chronic pancreatitis treatment, we propose to reconsider the etiological functions of progenitor-like ADM cells in chronic pancreatitis development from a new perspective and develop the new approach to cure chronic pancreatitis. In this reporting period, we finished the experiments to demonstrate that Vivace102 treatment could inhibit the pancreatic inflammation induced by YAP1/TAZ activation in pancreatic acinar cells. We also generated the new genetically modified mouse line and tested alcohol-dependent chronic pancreatitis induction in this line. We found that impairment of Hippo pathway in acinar cells increased the sensitivity of pancreas to alcohol treatment, but the incidence of alcohol-induced pancreatic fibrosis is still low. Our current data suggested that additional treatment to induce acute injury might help to establish a robust mouse model for alcohol-dependent chronic pancreatitis. We found that our genetically modified mice are sensitive to caerulein treatment-induced acute injury. We plan to incorporate caerulein treatment into our new chronic pancreatitis model in the next reporting period.

15. SUBJECT TERMS
Chronic pancreatitis, ADM, progenitor, fibrosis, YAP1, TAZ

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
U	U	U	UU	7	USAMRDC

Table of Contents

1. Introduction

Alcoholism is the major risk factor for chronic pancreatitis, but the molecular mechanisms promoting the development chronic pancreatitis in heavy drinkers remain unclear. To examine the hypothesis that progenitor-like ADM cells act as a targetable etiological factor for alcohol-dependent chronic pancreatitis, this project proposed to develop a new alcohol-dependent chronic pancreatitis model with genetically modified mice and test the therapeutic potential of a novel YAP1/TAZ inhibitor Vivace102.

2. Keywords

Chronic pancreatitis, alcohol, Vivace102, mouse model, Hippo pathway, Lats1&2, YAP1/TAZ

3. Accomplishments

What were the major goals of the project?

1) Develop a new alcohol-induced CP model to examine the effects of progenitor-like ADM cell accumulation on CP sensitivity. In the application, we proposed to finish two subtasks for this goal before Dec 2022. We have finished 70-80% of this goal in this reporting period;

2) Determine the effects of progenitor-like ADM cell accumulation on inducing fibrotic subpopulations of PSCs and macrophages. In the application, we proposed to start this goal in the second reporting period;

3) Evaluate whether Vivace102 treatment potential in preclinical model. We have finished the first subtask for this goal as proposed in the application. The second subtask will start from next reporting period.

What was accomplished under these goals?

The major activities in this reporting period include:

1) Generate genetically modified mouse lines needed in this project;

2) Optimize the alcohol feeding conditions for genetically modified mice to induce chronical pancreatitis;

3) Test the treatment conditions of Vivace102 in genetically modified mice.

The specific objectives are

1) Use the genetically modified mice to establish a new alcohol-dependent chronical pancreatitis model.

2) Test whether Vivace102 can suppress the pancreatic fibrosis induced by YAP1/TAZ activation in pancreatic acinar cells.

The significant results in this reporting period include:

1) We knocked out Lats1&2 in acinar cells by giving Ptf1a^{CreER} Lats1 $^{fl/fl}$Lats2 $^{fl/fl}$ mice single dose TAM IP injection to generate PL mice. Five day after the TAM administration, PL mice were randomly assigned into control group and vivace102 treatment groups. The Vivace102 was orally administrated once a day for 5 days at the concentrations of 2mg/kg and 3mg/kg. The control group was given saline. The pancreatic tissues were collected 1 day after the last Vivace102 or saline treatment for evaluation. As shown in the figure 1, the PL mice in Vivace102 treatment group showed significant less pancreatic tissue damage and inflammation than the PL mice in the control group treated with saline. We **concluded** that inhibition of persistent YAP1/TAZ activation suppressed the spontaneous pancreatic inflammation in PL mice, supporting that Vivace102 has the therapeutic potential for chronic pancreatitis treatment.

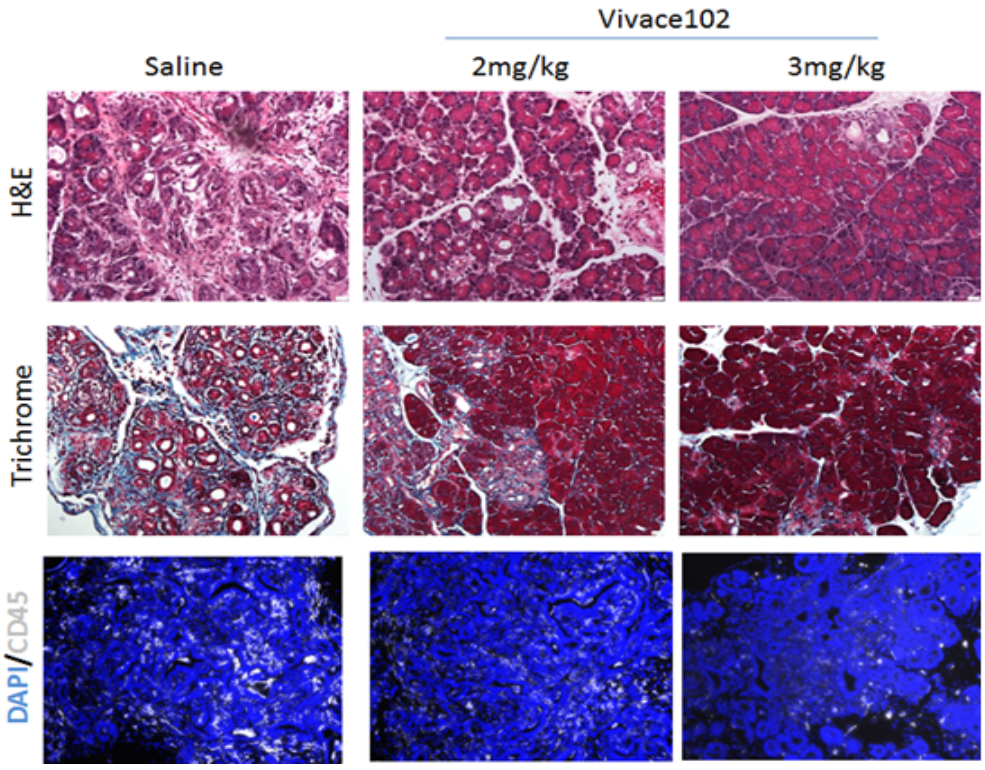

Figure1: Ptf1a^{CreER} Lats1$^{fl/fl}$ Lats2$^{fl/fl}$ mice were given single dose of TAM injection to delete Lats1&2 in acinar cells. At the day 6 of TAM injection, mice were orally administrated with saline or vivace102 for another 5 days. The pancreatic tissues were collected for H&E and Trichrome staining to analyze the histological alternations and collagen deposition. Immunofluorescent staining for CD45 was also applied to examine the infiltration of immune cells. The control group (n=6) treated with saline exhibited severe histological abnormalities, excessive collagen accumulation and large amount a infiltrated immune cells. The mice treated with 2mg/kg (n=6) or 3mg/kg (n=6) Vivace102 inhibited the pancreatic inflammation and fibrosis in does-dependent manner.

2) Ptf1a^{CreER} Lats1$^{fl/+}$Lats2 $^{fl/fl}$ mice were given 5 doses of TAM (180mg/kg) to disrupt three copies of Lats1/2 alleles in the acinar cells to generate of PL1^{Het}L2^{-} mice. Two weeks after the final TAM injection, the mice were fed with ethanol (Decon)-containing (5%, v/v) liquid diet (F1258SP; Bio-Serv) supplemented with maltose dextrin (Bio-Serv) for 4 weeks. The pancreatic tissues were harvested for histological evaluation. We found that ethanol-feeding alone did not induce the development of pancreatic fibrosis in PL1^{Het}L2^{-} mice. We have discussed this possibility in the application and proposed to give the mice additional single dose of alcohol binge drinking to induce acute injury in the pancreas. Initially, the mice were orally gavaged with ethanol (5 g/kg) three weeks after ethanol containing liquid diet. This protocol has been reported to induce mild pancreatic inflammation in a previous study. However, most of mice died within a few hours after the alcohol gavage administration in our pilot experiments. Thus, the amount of ethanol was reduced to 3g/kg in subsequent trials. This treatment was well tolerated by the mice. Although no obvious acute pancreatic injuries were observed in the pilot experiment (Figure 2A), we found that 4 of 11 PL1^{Het}L2^{-} mice exhibited pancreatic lesions with collagen accumulation 3 weeks after alcohol gavage administration (Figure 2B). We concluded that

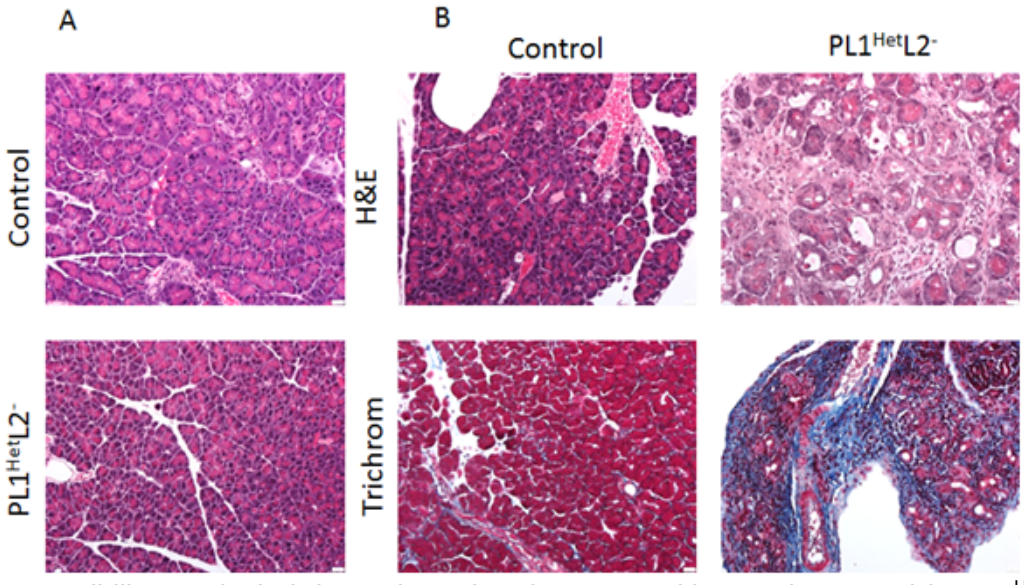

Figure2: We generate of PL1^{Het}L2^{-} mice to feed with ethanol -containing (4%, v/v) liquid diet for 3 weeks, followed by one time binge drinking of ethanol (3g/kg). (A) To analyze the acute pancreatic injuries with H&E staining, mice were sacrificed one day after alcohol binge drinking. We found that neither wild type mice (n=6) nor PL1^{Het}L2^{-} mice (n=6) exhibited obvious histological abnormalities; (B) one week after alcohol binge drinking, wild type control mice (n=9) still showed no significant histological abnormalities and collagen accumulation. But 4 of 11 PL1^{Het}L2^{-} mice developed pathological features of chronic pancreatitis ,according to the H&E and Trichrome staining

susceptibility to alcohol-dependent chronic pancreatitis was increased in PL1^{Het}L2^{-} mice, but alcohol feeding

did not consistently induce acute injuries in the pancreas of $PL1^{Het}L2^{-}$mice. Thus, to establish a robust alcohol-dependent chronic pancreatitis model, other methods that can efficiently induce acute pancreatic injury are needed in the future experiments. Caerulein treatment has been wildly used to induce acute pancreatic injury in mouse models. In another research project, we recently found that $PL1^{Het}L2^{-}$ mice are highly sensitive to caerulein treatment. The $PL1^{Het}L2^{-}$ mice developed more ADM lesions and showed delayed tissue repair after caerulein treatment (figure 3). This observation supports our hypothesis that accumulation of ADM cells in $PL1^{Het}L2^{-}$mice may act as an etiological factor to promote chronic pancreatitis progression. Thus, we predict that $PL1^{Het}L2^{-}$ mice will be more susceptible to alcohol-induced chronic pancreatitis development after acute caerulein treatment. Previous study reported that repeated caerulein injection for 3 weeks is required to induce alcohol-dependent chronic pancreatitis. We will test whether caerulein injection for 1 day is sufficient to induce alcohol-dependent chronic pancreatitis in $PL1^{Het}L2^{-}$ mice.

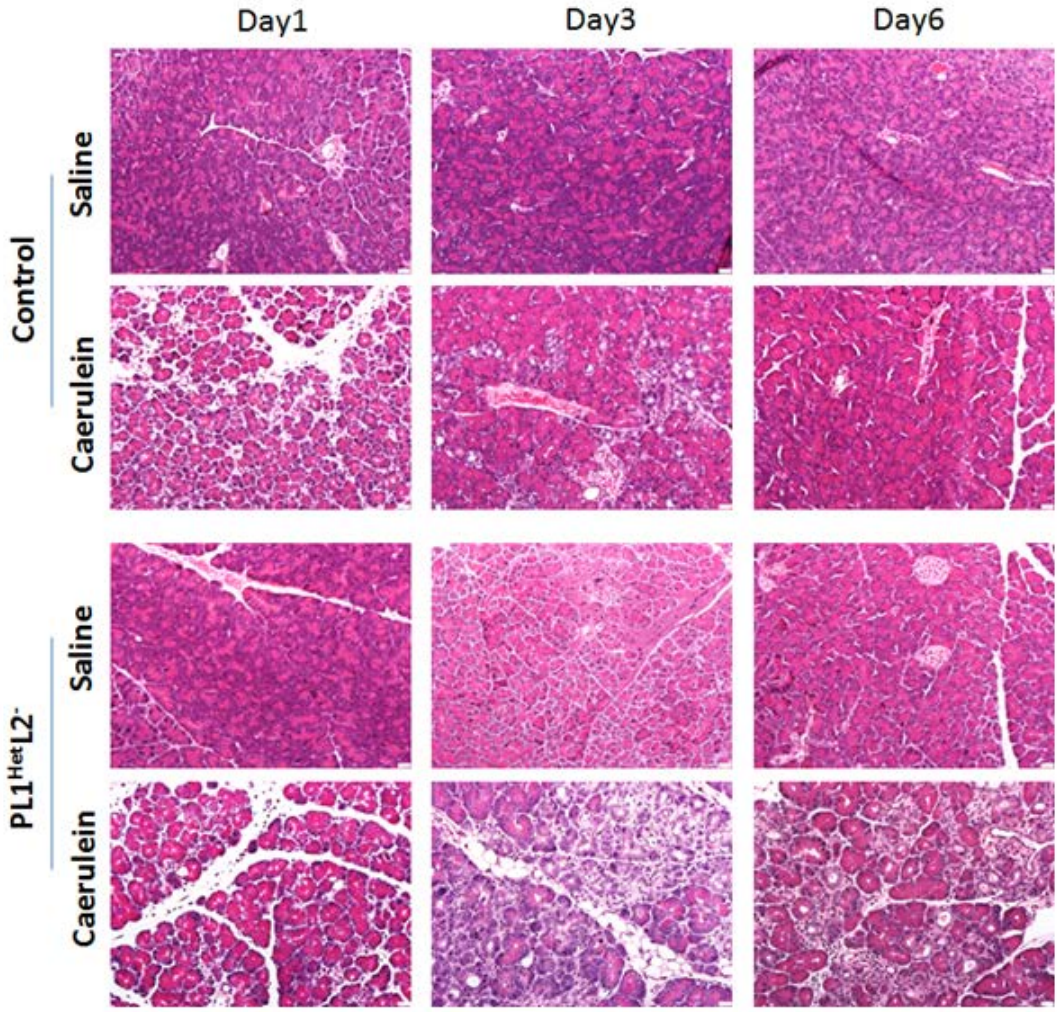

Figure3: We compared the sensitivity of wild type control mice and $PL1^{Het}L2^{-}$ mice to caerulein -induced acute pancreatic injuries. Saline or caerulein (50 µg/kg) was administered through 8 i.p. injections at hourly intervals per day for 2 days. Pancreatic injuries were assessed by HE staining on days 1, 3 and 6 after the final injections. We found that saline injection did not cause pancreas damages in wild type control mice (n=4) and $PL1^{Het}L2^{-}$ mice (n=6) at all time points examined. Caerulein treatment induced acute injuries in wild type mice (n=6), which were almost fully repaired at day 6 after caerulein treatment. Caerulein treatment also constantly induced acute pancreatic injuries in $PL1^{Het}L2^{-}$ mice (n=6). Histological evaluation revealed that $PL1^{Het}L2^{-}$ mice developed more ADM lesions at the day3 after caerulein treatment than the wild type control mice. In addition, some ADM lesions still persisted even at the day6 after caerulein treatment in $PL1^{Het}L2^{-}$ mice.

What opportunities for professional development has the project provided? Nothing to Report

How were the results disseminated to communities of interest? Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?

In the next reporting period, we will finish the statistical analysis for the data generated in the current reporting period. At the same time, we plan to combine the caerulein treatment and alcohol feeding to establish a robust model for alcohol-dependent chronic pancreatitis model with our genetically modified mice, which will finish the major task 1 in the application. With the new model, we will start the research for the major task2 in our application by using the single RNA-seq and 3D culture system to determine the effects of progenitor-like ADM cell accumulation on inducing fibrotic subpopulations of PSCs and macrophages. Based on the results

obtained in this reporting period, we will test whether Vivace102 can improve the alcohol-dependent pancreatitis in the new mouse model to finish the major task 3.

4. Impact

What was the impact on the development of the principal discipline(s) of the project?

Chronic pancreatitis is a progressive disease which causes permanent pancreas damage leading to food maldigestion. We still do not understand the reasons for CP development at molecular level and there is not effective treatment for CP. A previous study demonstrated that Hippo pathway inactivation in acinar cells induced pancreatic inflammation and fibrosis in YAP1/TAZ dependent manner. Our current data demonstrated that treatment with Vivace102, a new YAP1/TAZ inhibitor, can suppress the spontaneous pancreatic inflammation and fibrosis in mice with Hippo pathway inactivation. Since Hippo pathway insufficiency is closely associated with CP development, our results support that pharmaceutically targeting the key effectors in Hippo pathway might be a promising strategy to treatment CP.

What was the impact on other disciplines? Nothing to Report

What was the impact on technology transfer? Nothing to Report

What was the impact on society beyond science and technology? Nothing to Report

5. Changes/Problems

Nothing to Report

6. Products

Nothing to Report

7. Participants & Other Collaborating Organizations

What individuals have worked on the project? Jun Liu/ no change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period? Nothing to Report

What other organizations were involved as partners? Nothing to Report

8. Special Reporting Requirements

Nothing to Report

9. Appendices