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TITLE: Proinflammatory Epithelial Cells as a Therapeutic Target in Chronic Pancreatitis

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14. ABSTRACT

The focus of our study is the role of de-differentiated exocrine acinar cells in perpetuating inflammation and tissue injury in chronic pancreatitis. We hypothesize that the pro-differentiation transcription factor Ptf1a promotes recovery from acute pancreatitis, in part through secretion of anti-inflammatory signals such as FGF21, and that downregulation of Ptf1a in chronic pancreatitis leads to sustained inflammation. We have made only limited progress, unfortunately, although the personnel issues that affected us in the beginning of the project have been resolved. We have a large series of experimental mouse samples harvested and fixed, ready to analyze as work on campus begins to resume (we have moved to a partially-open status this month).

15. SUBJECT TERMS
pancreatitis, pancreas, exocrine, acinar, inflammation, differentiation, Ptf1a, FGF21

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

   The subject of this proposal is chronic pancreatitis, an inflammatory condition of the pancreas that is debilitating and untreatable. We hypothesize that persistent inflammation in this disease is driven in part by signals from residual epithelial cells that form via de-differentiation of exocrine acinar cells, the majority cell type in the organ. In mouse models, we propose to determine if the pro-differentiation transcription factor Ptf1a and its downstream target gene FGF21 act to inhibit inflammation by stabilizing the differentiated state.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

   pancreatitis, pancreas, exocrine, acinar, inflammation, differentiation, Ptf1a, FGF21

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

   What were the major goals of the project?
   List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

   Aim 1. Determine if PTF1A expression is sufficient to revert ADM cells to a re-differentiated state and resolve inflammation and tissue injury in a chronic pancreatitis model.

   Major Task 1. Establish *Ptf1a-cKO/GOF* model, determine effects of re-expressing PTF1A on chronic pancreatitis-like phenotype

   Months 1-14 – 25% complete

   Aim 2. Determine if exogenous FGF21 administration is sufficient to revert the pro-inflammatory phenotype of ADM cells, and resolve tissue injury, independent of re-differentiation.

   Major Task 2. Determine effects of FGF21-Fc on chronic-pancreatitis-like phenotype of Ptf1a-cKO mice

   Months 1-18 – 50% complete

   Aim 3. Identify markers of ADM cells in mouse pancreas, and characterize their expression in human chronic pancreatitis.

   Major Task 3. Pilot studies to optimize FACS isolation of specific cell types

   Months 4-8 – 75% complete

   Major Task 4. RNA-seq analysis of acinar, duct and ADM-specific gene expression

   Months 1-13 – 25% complete

   Major Task 5. Analyzing protein marker expression in mouse and human chronic pancreatitis

   Months 13-18 – 0% complete

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Major activities
Progress on this project was initially delayed due to personnel turnover, but my new graduate student working on the project is now fully up to speed on the methodology, and in addition I was able to hire a full-time technician in early 2020 who will be working on this project. Before the COVID-19 shutdown in March, my student harvested and fixed samples from about half of the mice needed for Aim 2 of the project. Breeding for the mice in Aim 1 was delayed for technical reasons, and then postponed for COVID-19, but those crosses should yield useable offspring in the next 2-3 months. In the meantime, my student has developed a new approach for quantitative imaging of our histological sections, using slide-scanning microscopy, and is working on integrating this technique with machine-learning tools that have been developed for quantitative tissue analysis.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

This project is largely being driven by a graduate student in the Murtaugh lab, Diane Hernandez, who has presented her work in departmental student research seminars, at our annual retreat, and in a research seminar organized by the Training Program in Genetics, an NIH training grant on which she was awarded as fellowship position. Diane has mentored two undergraduate students, one of whom, Ella Gregory, has helped her develop her histology analysis methods. Due to the COVID-19 campus shutdown, Ms. Gregory is currently unable to work in the lab.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

The PI presented findings related to chronic pancreatitis in *Ptf1a* cKO mice, and discussed the potential for rescue by Ptf1a re-expression or FGF21 treatment, at meetings including the Gordon Research Conference on Pancreatic Diseases (2019) and the Nature Transdisciplinary Cancer Interception conference (2020), and seminars including Baylor College of Medicine (2019). No other public outreach per se has been undertaken yet.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Aim 1. Re-express Ptf1a after establishing chronic pancreatitis in the *Ptf1a* cKO/caerulein model (Fig. 1D), using a DOX-dependent *tetO-Ptf1a* transgene, and determine if this is sufficient to resolve inflammation and injury. We have recently published on the efficacy of this transgene:
Krah et al. [2019] *Developmental Cell* 50: 744-754; doi.org/10.1016/j.devcel.2019.07.012t

Aim 2. Administer FGF21 after establishing chronic pancreatitis in the *Ptf1a* cKO/caerulein model, determine if this is sufficient to resolve inflammation and injury.

Aim 3. FACS isolate dedifferentiated acinar cells from the *Ptf1a* cKO/caerulein model, perform RNA-seq to identify changes in gene expression relative to controls. Using antibodies against select upregulated gene products in mouse, analyze whether similar upregulation occurs in human chronic pancreatitis.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

At this point we have not achieved major accomplishments. Our preliminary data has been well-received in the field, and we anticipate that our findings will be of interest to the pancreatitis field similar to our pancreatic cancer work recently published in *Developmental Cell*.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are*

significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

We encountered delays in this work due first to turnover in personnel, and more recently due to the COVID-19 campus shutdown. While keeping spending of the award to a minimum, we were able to establish highly-reproducible conditions for the *Ptf1a* cKO/caerulein model of chronic pancreatitis, with training of relevant personnel. We have also hired a new full-time technician, whose responsibilities will encompass this work when he is able to return to the lab.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Overall spending has been very constrained; in addition to losing the technician who was working in the lab when the grant period started, my student Diane Hernandez won a training grant fellowship that has covered her salary. We have been focusing on experimental optimization rather than the large scale of mouse breeding and analysis anticipated in our proposal.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Not applicable (no human subjects).

Significant changes in use or care of vertebrate animals

No changes. Our current animal protocol, 19-10003, was approved by the University of Utah IACUC on October 22, 2019

Significant changes in use of biohazards and/or select agents

Not applicable (no biohazards or select agents).

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to report.

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to report.

  Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

"Ptf1a and acinar cell programming, reprogramming and re-reprogramming." Gordon Research Conference on Pancreatic Diseases, June 20, 2019. Invited presentation by Dr. Murtaugh.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

  Nothing to report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

  Nothing to report.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

  Nothing to report.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*

- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name:	Lewis Charles Murtaugh
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	LCMURTAUGH (NIH eCommons)
Nearest person month worked:	3
Contribution to Project:	Designed and led project including assistance with training and trouble-shooting.
Funding Support:	National Institutes of Health (2R01DK061220-11A1, 5R01CA194941-04)

Name:	Diane Hernandez
Project Role:	graduate student
Researcher Identifier (e.g. ORCID ID):	DHERNANDEZ37 (NIH eCommons)
Nearest person month worked:	6
Contribution to Project:	Mouse breeding and experimentation.
Funding Support:	Training Program in Genetics, NIH 5T32GM007464

Name:	Julie Ann Straley
Project Role:	technician
Researcher Identifier (e.g. ORCID ID):	n/a
Nearest person month worked:	3
Contribution to Project:	Mouse breeding.
Funding Support:	National Institutes of Health (2R01DK061220-11A1, 5R01CA194941-04)

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to Report.