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14. ABSTRACT To better understand the role of tuft cells in the pancreas and to define their effects on the pancreatic body as well as the initiation of pancreatic cancer. Tuft cells are present in the hollow organs of the digestive and respiratory tracts. They are characterized by long and blunt microvilli with prominent rootlets and by a well-developed tubulovesicular system in the supranuclear cytoplasm. Recent reports suggest that tuft cells may act as mechanoreceptors and are involved in chemosensing of the microenvironment. With the successful deletion of Dclk1 throughout the pancreatic ducts, we have characterized, which extends our understanding of the role tuft cells play within ducts and their broader effects on the overall pancreatic microenvironment. We observed that Dclk1 co-localized with other putative tuft cell markers including Cox1 and Cox2. Additionally, we also observed the presence of the tuft cells of wild type and <i>Pdx-1-Cre;Dclk1^{flox/flox}</i> mice, indicating that Dclk1 may not play a role in tuft cells at baseline. We performed additional experiments to demonstrate the role of Dclk1 in pancreatic inflammation leading to pancreatic neoplasia. Dclk1 played a vital role in governing and is essential for pancreatic inflammatory process and associated metaplastic progression following caerulein-induced acinar ductal metaplasia. These finding could very well provide the basis for the development of novel chemotherapeutic drugs targeting these specialized cells expressing Dclk1 for eradication of pancreatitis and pancreatic adenocarcinoma. The data generated from this DoD grant played a major role in securing a successful R01 (1R01 CA182869 01A1), one scientific research publication (<i>PLoS One</i> . 2015 Feb 27;10(2):e0118933), and one scientific presentation at the 2014 American Pancreatic Association conference (<i>Pancreas</i> . 2014 43(8):1389-90).					
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INTRODUCTION: To better understand the role of tuft cells in the pancreas and to define their effects on the pancreatic body as well as the initiation of pancreatic cancer. Tuft cells are present in the hollow organs of the digestive and respiratory tracts. They are characterized by long and blunt microvilli with prominent rootlets and by a well-developed tubulovesicular system in the supranuclear cytoplasm. Recent reports suggest that tuft cells may act as mechanoreceptors and are involved in chemosensing of the microenvironment.

BODY:

Aim/Task 1: To characterize tuft cell expression in the mouse pancreas. (1 – 12 months)

Tasks 1a and b: The initial breeding for the generation of the proposed *p48^{cre};KRAS^{G12D}* mouse model proved problematic and the rate of genotypically desirable offspring was not consistent with expected and proposed results. The animal numbers needed would not be reached at an appropriate time for the completion of proposed work. Therefore we sought to replace the current *p48^{cre}* breeding stock with newly acquired *Pdx-1-Cre;KRAS^{G12D}* mice. As per the proposal, we characterized the tuft cells in pancreatic tissues in the presence and absence of *Dclk1*. In the wild type mice, we did find the co-localization of *Dclk1* with *Cox1*, *Cox2*, and other tuft cell markers (Figure 1) in all age groups.

Aim/Task 2: To determine the role of *Dclk1* (DCAMKL-1) in pancreatic tuft cell regulation and regulation of miRNAs in tuft cells. (1 – 12 months)

Task 2a: Here we have successfully crossed *Pdx-1-Cre* to *Dclk1^{flox/flox}* (formally DCAMKL-1) and created the novel compound mouse model *Pdx-1-Cre;Dclk1^{flox/flox}* (*Dclk1*-KO mice).

We performed experiments and confirmed that *Dclk1* was in fact deleted from all pancreatic ductal regions as expected from utilizing the *Pdx-1-Cre* promoter (Figure 1). We have also characterized the expression of other tuft cell markers *Cox1* and *Cox2* (Figure 1). As said above, in wild type mice there was co-localization of *Dclk1* with *Cox1* and *Cox2*. Whereas in *Dclk1*-KO mice, we still found the presence of other tuft cell markers *Cox1* and *Cox2* indicating that tuft cells exist in the absence of *Dclk1*. Furthermore, in the immunoelectron microscopy, we did not find any differences in the tuft cells with *Dclk1* or absence of *Dclk1* (*Dclk1*-KO mice). Additionally, also observed the presence of tuft cells in *Dclk1*-KO mice with *KRAS* mutation. This indicates that the absence of *Dclk1* embryonically did not change the outcome/nature of the tuft cell.

Task 2b: Following the recent successful creation and characterization of the *Pdx-1-Cre;Dclk1^{flox/flox}* compound mice, completion of this task was achieved. mRNA and miRNA analysis utilizing RT-PCR has been completed for the control mice (WT) and *Dclk1*-KO mice, we confirmed the absence of *Dclk1* mRNA in the mouse pancreas (Data not shown). We performed miRNA analysis of *Dclk1*⁺ tuft cells from C57BL/6 wild type mice as proposed in our application. Cells of the pancreata were isolated using

Alexa Fluor conjugated Dclk1 antibody and FACS sorted into ve^+ and ve^- subpopulations. These subpopulations were then subjected to RT-PCR analysis. We found that Dclk1+ cells had less tumor suppressor miRNAs *let-7a*, *miR-144*, and *miR-200a* (Figure 2). No difference in the ultrastructural investigation of these cell subpopulations was found.

Aim 3: To determine the mechanism by which DCAMKL-1+ tuft cells regulate pancreatic cancer initiation. (12 – 24 months)

For this aim we utilized novel mouse line *Pdx-1-Cre;Dclk1^{flox/flox}* with the *KRAS^{LSL-G12D}*. We employed mice from different age groups and compared the pancreatic initiation profile. Profiling included the number and size of the tumors. We expected the *Pdx-1-Cre;KRAS^{LSL-G12D}* mice will develop rapid pancreatic tumors compared to *Pdx-1-Cre;KRAS^{LSL-G12D};Dclk1^{flox/flox}* mice. We observed that the pancreatic initiation in both the mouse lines was around 10 months and we did not find any difference between the two groups. This is in-line with the previous published Nature Genetics article where *Apc^{min/+}* mice with Dclk1 knockout did not show any differences with the polyp or tumor formation [4]. These data taken together indicate that embryonic deletion of Dclk1 does not have any role in pancreatic cancer initiation and progression. Alternatively, another mutation (p53) is required for hastening the cancer initiation process (we have proposed these methods in our newly awarded R01, which is in continuous of our DoD grant proposal.) ***The preliminary data obtained from this DoD grant played a crucial role in securing the newly awarded R01 (1R01 CA182869 01A1).***

KEY RESEARCH ACCOMPLISHMENTS:

- Successful creation of the novel compound mouse *Pdx-1-Cre;Dclk1^{flox/flox}*.
- Initial characterization of *Pdx-1-Cre;Dclk1^{flox/flox}* was completed which demonstrated the loss of Dclk1 expression within tuft cells in all pancreatic ducts.
- Successful creation of the novel compound mouse *Pdx-1-Cre;KRAS^{LSL-G12D}*
- Successful creation and characterization of novel compound mouse Created the novel compound mouse *Pdx-1-Cre;KRAS^{LSL-G12D}; Dclk1^{flox/flox}*

REPORTABLE OUTCOMES:

- Created the novel compound mouse *Pdx-1-Cre;Dclk1^{flox/flox}*
- Created the novel compound mouse *Pdx-1-Cre;KRAS^{LSL-G12D}*
- Created the novel compound mouse *Pdx-1-Cre;KRAS^{LSL-G12D}; Dclk1^{flox/flox}*
- The data generated from this DoD grant played a major role in securing a successful R01 grant application (**1R01 CA182869 01A1** – The role of DCLK1 in the initiation of pancreatic ductal adenocarcinoma, **PI: Courtney W. Houchen**, April, 2015 – Mar, 2020).
- Successfully published recently in journal PLoS ONE (**Qu et al., PLoS One. 2015 Feb 27;10(2):e0118933**) [5].

CONCLUSION:

With the successful deletion of Dclk1 throughout the pancreatic ducts, we have characterized, which extends our understanding of the role tuft cells play within ducts and their broader effects on the overall pancreatic microenvironment. We found that Dclk1 co-localizes with other potential tuft cell markers like Cox1, and Cox2 in pancreatic tissues. There is existence of tuft cells in the absence of Dclk1 indicating that at baseline or normal conditions, Dclk1 may not play a role in tuft cells (this is similar to our previous observation that Dclk1 marks a quiescent intestinal stem/tuft cells). In this DoD grant application, we discovered that Dclk1 had a limited role in pancreatic cancer initiation in an embryonically deleted Dclk1 with KRAS mutation. This lead to the conclusion that another mutation (p53) may be required for the initiation of pancreatic cancer and Dclk1 may play a role in pancreatic initiation in the double mutant mice. These studies are proposed in our newly secured R01 grant application.

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APPENDICES: None

SUPPORTING DATA:

