AWARD NUMBER: W81XWH-18-1-0495

TITLE: Investigating the Role of Piezo1 in Pancreatic Cancer-Related Immune Suppression and Disease Progression

PRINCIPAL INVESTIGATOR: George Miller

CONTRACTING ORGANIZATION: NYU School of Medicine
New York, NY 10016

REPORT DATE: September 2019

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
Sept 2019	Annual Report	15 Aug 2018 - 14 Aug 2019

4. TITLE AND SUBTITLE

Investigating the Role of Piezo1 in Pancreatic Cancer-Related Immune Suppression and Disease Progression

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-18-1-0495

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
George Miller

E-Mail: George.miller@nyumc.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

New York University School of Medicine
550 First Ave.
New York, NY 10016

8. PERFORMING ORGANIZATION REPORT NUMBER

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

U.S. Army Medical Research and Materiel 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

Tumor stiffness and peri-tumoral fibrosis have emerged as factors limiting therapy for pancreatic ductal adenocarcinoma (PDA). Novel drugs directly targeting tumor stiffness have shown efficacy in preclinical studies and early clinical trials in pancreatic cancer. Piezo1 is a recently-discovered mechano-sensitive ion channel that governs organogenesis in response to physical pressure. Since PDA is subject to the physical pressures associated with progressive fibrosis, we postulated a role for Piezo1 in regulating disease progression. We found marked upregulation of Piezo1 in PDA. We also found that blocking Piezo1 signaling *in vivo* in PDA is protective and activates T cells and redirects myeloid cellular differentiation away from immune-suppressive subsets and protects against PDA. By contrast, activating Piezo1 promotes PDA growth and the accumulation of immune-suppressive monocytic cells.

15. SUBJECT TERMS
Piezo1, T cells, Cancer

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

Table of Contents

Page

Introduction

We discovered that Piezo1 is expressed in pancreatic dutal adenocarcinoma (PDA). We showed that pan-inhibition or compartment-specific deletion of Piezo1 is tumor-protective whereas Piezo1 activation accelerates tumorigenesis. Our cellular studies showed that Piezo1 alters both the myeloid cell and T cell programming in PDA.

Keywords

Piezo1, T cells, Cancer

Accomplishments

- **What were the major goals of the project?**

Aim 1. To determine the effects of global and compartment-specific inhibition or activation of Piezo1 on PDA progression

Aim 2. To determine the mechanistic influence of Piezo1 on monocytic cellular differentiation in the PDA tumor microenvironment

Aim 3. To determine whether targeting Peizo1 enables efficacy of checkpoint-based immunotherapy in PDA

- **What was accomplished under these goals?**

Major activities: (i) In vitro studies of Piezo1 expression in PDA, **(ii)** In vivo tumor experiments and analysis of immune changes when modulating Piezo1

Specific Objectives:

Major task 1: Investigate immune-suppressive differentiation of intra-tumoral stiffness induced Piezo1 in PDA in three distinct mouse models of PDA and in human disease

Subtask 1: To examine Piezo1 expression in PDA

Subtask 2: Targeting Piezo1 in PDA

Subtask 3: Dependence of tumor-protection of reducing tumor-stiffness on Piezo1

Major Task 2: Investigate how targeting Piezo1 affects monocytic cell expansion, differentiation, and their capacity to affect immunogenic T cell differentiation.

Subtask 1: Pivotal role of Piezo1 signaling in expansion of immune-suppressive TAMs in PDA

Subtask 2: Central role of Piezo1 signaling in expansion of MDSC in PDA

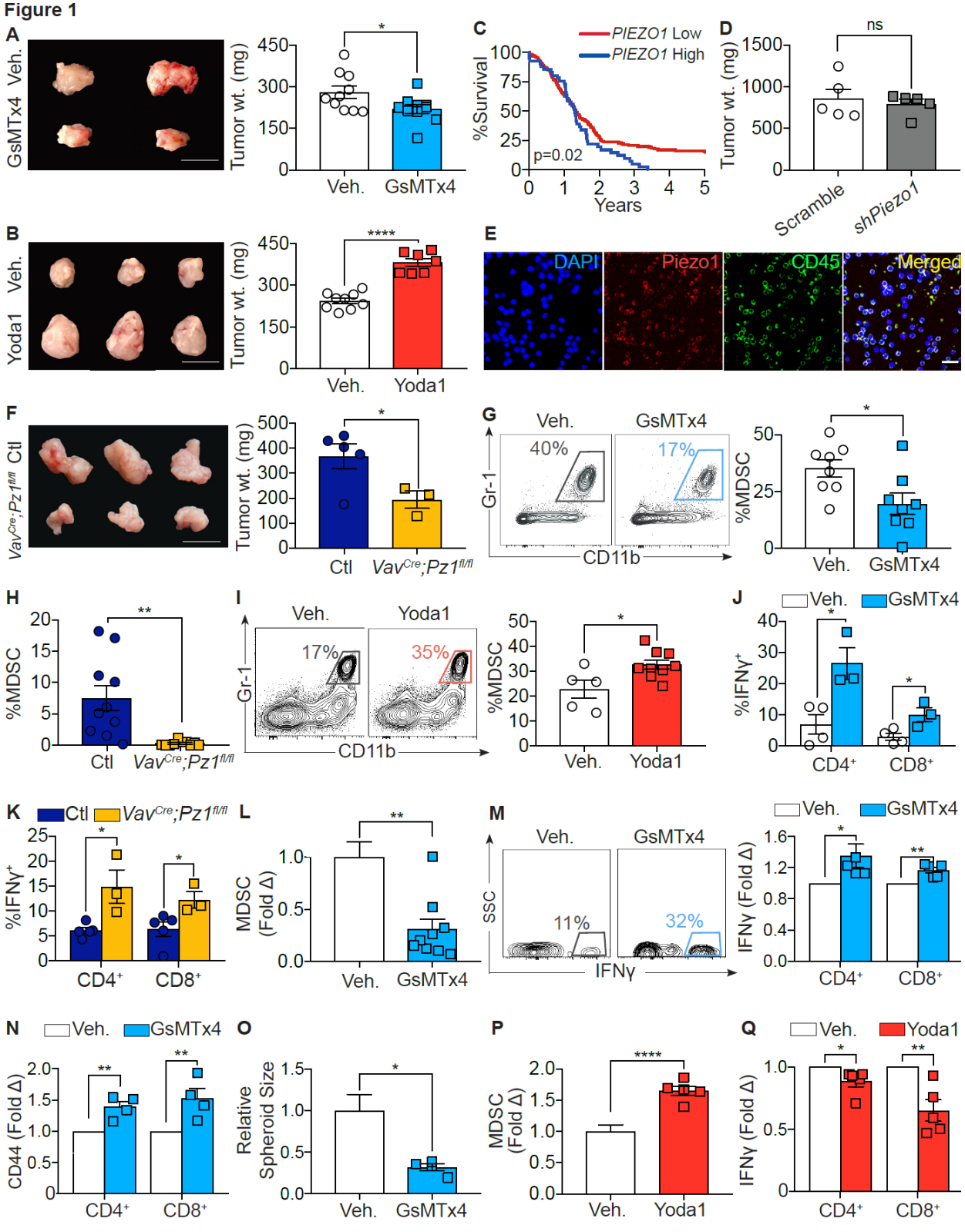
Significant results or key outcomes:

To assess the influence of global Piezo1 signaling on the progression of pancreatic ductal adenocarcinoma (PDA), we utilized GsMTx4 and Yoda1, respectively, to inhibit or activate Piezo1. Inhibition of Piezo1 conferred tumor-protection in an orthotopic PDA model using tumor cells derived from *Pdx1*^{Cre}*;Kras*^{G12D}*;Tp53*^{R172H} (KPC) mice (**Figure 1A**). By contrast, activation of Piezo1 accelerated tumor growth (**Figure 1B**). Similarly, in human PDA, low *PIEZO1* expression was associated with extended survival (**Figure 1C**). Whereas ~20% of patients that expressed low *PIEZO1* were 5-year survivors, there were no long-term survivors in the *PIEZO1* high group. PDA tumor cells expressed Piezo1 (**Figure S1A**); however, neither activation nor inhibition of Piezo1 affected tumor cell growth *in vitro* (**Figure S1B**), nor did knockdown of *Piezo1* affect PDA growth *in vivo* (**Figures 1D, S1C**). We therefore postulated that inhibiting Piezo1 mitigates oncogenic progression by reprograming the inflammatory tumor

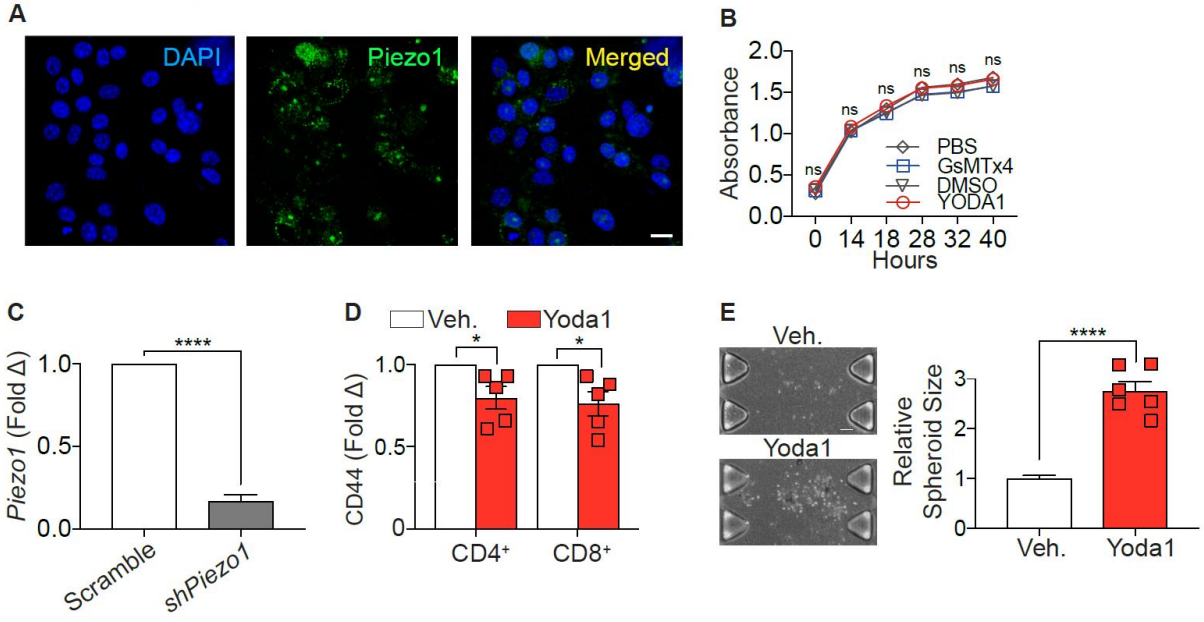
microenvironment (TME). Using *Piezo1*$^{P1\text{-}tdT}$ mice that express a fluorescent tdTomato reporter from the *Piezo1* promoter, we confirmed that leukocytes robustly express Piezo1 (**Figure 1E**). To directly test our hypothesis, we generated *Vav*^{Cre}*;Piezo1*$^{fl/fl}$ mice in which *Piezo1* is deleted from all hematopoietic cells. *Vav*^{Cre}*;Piezo1*$^{fl/fl}$ mice demonstrated attenuated tumor growth compared to littermate controls (**Figure 1F**).

We analyzed the intra-tumoral immune phenotype in PDA-bearing mice treated with GsMTx4. We discovered that the Gr1^{+}CD11b^{+} myeloid-derived suppressor cell (MDSC) population was sharply diminished upon Piezo1 inhibition (**Figure 1G**). Similarly, PDA tumors in *Vav*^{Cre}*;Piezo1*$^{fl/fl}$ mice exhibited lower MDSC infiltration (**Figure 1H**). By contrast, activation of Piezo1 *in vivo* with Yoda1 increased MDSC expansion in PDA (**Figure 1I**). Furthermore, consistent with immunogenic reprogramming in the TME, CD4^{+} and CD8^{+} T cells upregulated IFNγ expression in PDA tumors in WT hosts treated with GsMTx4 (**Figure 1J**), as well as in PDA tumors in *Vav*^{Cre}*;Piezo1*$^{fl/fl}$ mice (**Figure 1K**), indicative of adaptive immune activation. To evaluate the therapeutic efficacy of Piezo1 inhibition in human PDA, we treated patient-derived organotypic tumor spheroids (PDOTS) from freshly resected human tumors with GsMTx4 or vehicle using a 3-dimensional microfluidic system we recently validated as a platform for testing immune-based therapeutics. Piezo1 inhibition resulted in MDSC contraction, CD4^{+} and CD8^{+} T cell activation, and attenuated spheroid growth in PDOTS (**Figures 1L-O**). By contrast, Piezo1 activation expanded MDSC, aggravated T cell suppression, and promoted spheroid growth in PDOTS (**Figures 1P, Q, S1D, E**).

Figure 1


A
Veh.
GsMTx4
Tumor wt. (mg)
Veh.
GsMTx4
B
Veh.
Yoda1
Tumor wt. (mg)
Veh.
Yoda1
C
PIEZO1 Low
PIEZO1 High
%Survival
p=0.02
Years
D
ns
Tumor wt. (mg)
Scramble
shPiezo1
E
DAPI
Piezo1
CD45
Merged
F
Ctl
Vav^Cre;Pz1^fl/fl
Tumor wt. (mg)
Ctl
Vav^Cre;Pz1^fl/fl
G
Veh.
GsMTx4
40%
17%
Gr-1
CD11b
%MDSC
Veh.
GsMTx4
H
%MDSC
Ctl
Vav^Cre;Pz1^fl/fl
I
Veh.
Yoda1
17%
35%
Gr-1
CD11b
%MDSC
Veh.
Yoda1
J
Veh.
GsMTx4
%IFNγ+
CD4+
CD8+
K
Ctl
Vav^Cre;Pz1^fl/fl
%IFNγ+
CD4+
CD8+
L
MDSC (Fold Δ)
Veh.
GsMTx4
M
Veh.
GsMTx4
11%
32%
SSC
IFNγ
Veh.
GsMTx4
IFNγ (Fold Δ)
CD4+
CD8+
N
Veh.
GsMTx4
CD44 (Fold Δ)
CD4+
CD8+
O
Relative Spheroid Size
Veh.
GsMTx4
P
MDSC (Fold Δ)
Veh.
Yoda1
Q
Veh.
Yoda1
IFNγ (Fold Δ)
CD4+
CD8+

Supplemental Figure 1



- **What opportunities for training and 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?**

We plan to submit manuscript and present at national conference.

Impact

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

Nothing to Report.

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.

Changes/Problems

Nothing to Report

Products

Nothing to Report

Participants and other collaborating organizations

- **What individuals have worked on the project?**

  George Miller, PI

  Berk Aykut, Postdoctoral Fellow

  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.

Special reporting requirements

Not applicable

Appendices

None