Microbial Programming of the Tumor Microenvironment

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Disclosures

- RCA with GSK
- Co-Founder NYBO Therapeutics
Inflammatory Context of Pancreatic Cancer

Feig et al., Clin Can Res 2012

Inflammatory Context of Pancreatic Cancer

Mallen-St.Clair et al., Genes & Development 2012
**Inflammation is a Precursor to Dysplasia**

![Histological images showing progression from normal tissue to dysplastic tissue over time.](image1)

**Inflammation is Necessary for Pancreatic Oncogenesis**

![Diagram illustrating the relationship between inflammation and pancreatic cancer development.](image2)

**Chronic Pancreatitis Is Essential for Induction of Pancreatic Ductal Adenocarcinoma by K-Ras Oncogenes in Adult Mice**

Cancer Cell Article: Chronic Pancreatitis Is Essential for Induction of Pancreatic Ductal Adenocarcinoma by K-Ras Oncogenes in Adult Mice

Cancer Cell 71, 291-302, March 2007

**Significance**

Human PanINs and PDA have been faithfully reproduced in mouse models by expressing an endogenous K-Ras oncogene in pancreatic lineages during embryonic development. Here, we describe a mouse model that allows controlled temporal expression of an endogenous K-Ras oncogene in cells of acinar and ductal origin. These mice develop the full spectrum of PanINs and invasive PDA when K-Ras expression is allowed during embryonic development. Surprisingly, K-Ras expression in adult mice does not result in neoplastic development unless they undergo chronic pancreatitis. Previous epidemiological studies have identified pancreatitis as a risk factor for human PDA. Thus, close monitoring of people who may have suffered pancreatic tissue damage may help to identify PDA patients in the early stages of the disease.
Why has pancreatic cancer immunotherapy failed?

Cancer Immunology

- Innate Immune cells: Macrophages
- Adaptive Immune cells: T cells
- Activating Signals
- Inhibitory Signals
- Tumor Lysis or Tolerance
Why Has Immunotherapy Failed in Pancreas Cancer Treatment?

- Few
- Inactivated
- Th2

**Intracellular Immune cells**

- Macrophages

**Adaptive Immune cells**

- T cells

**Inhibitory Signals**

- Tolerance

**UNPRODUCTIVE IMMUNOTHERAPY**

**Chemokines**

- CCL2
- CCL5
- CXCL1
- CXCL5
- CXCL12
- CXCL13

**Toll-like receptors**

- TAM

**Chemokine receptors**

- CCR2

**Cytokines**

- IL-1β
- IL-10
- IL-4
- IL-6
- IL-8
- TNF

**DAMPs**

- HMGB1
- S100A9
- SAP130

**T cell receptor**

- αβ
- γδ

**Growth factors**

- TGF-β
- GM-CSF

**MDSC**

**CD8**

**Zambiris & Miller, Trends in Mol Med**
Introduction to Shared Resources

Pancreas Cancer Immunotherapy: From Basic Discovery to Development of Clinical Trials & New Therapeutics

Design of α-γδ T cell Ab
NYBO Therapeutics

Design of αGAL-9 Ab
NYBO Therapeutics

Nature Medicine 2017

Cell 2016

Immune suppression

No Apoptosis

Pre-Clinical Development

Innovative Clinical Trials

New Immune-based Therapeutics

Immunotherapy Pipeline
Pancreas Cancer Immunotherapy: From Basic Discovery to Development of Clinical Trials & New Therapeutics

**Immune suppression**

- **TLR ligands**
- **Bacteria**
- **RIP 1/3 Cell Death**
- **CXCL1**
- **M-CSF**
- **Nature 2016 Clinical Trial (RIP1 Inhibitor)**
- **Gastroenterology 2016 Clinical Trial (RT+ αM-CSFR)**

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A growing body of clinical evidence has uncovered links between the microbiota and the Hallmarks of Cancer. These include butyrate, a short-chain fatty acid; colibactin, a genotoxin; and FeSa and Fap2, bacterial mechanisms of *Fusobacterium nucleatum*. For further reading, please visit [this link](#).
Hypothesis

In genetically susceptible hosts the microbiome alters the risk of pancreatic carcinogenesis and can promote aggressive disease biology.

Question

Can Gut Bacteria Access the Pancreas?
Gut Bacteria can access the pancreas

![GFP-labeled E.coli: 40x](image1)

GFP+ Foci/HPF

Control

GFP

**

Gut bacteria can access the pancreas

![Diagram of the pancreas](image2)
Experimental Question

• Is the pancreatic microbiome altered in PDA?

Markedly more abundant intra-pancreatic microbiome in human PDA

NML; 20x  PDA; 20x
Markedly more abundant intra-pancreatic microbiome in human PDA

![Graph showing bacterial DNA levels in NML and PDA]

Mouse Models of PDA

- "KC" – Kras, pre-invasive
- "KPC" – Kras + p53, invasive
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1000X Abundant Intra-Pancreatic Microbiome in Murine PDA

![Graph showing bacterial DNA (pg) comparison between WT and KC mice.]

Comparison of Pancreatic Microbiome in Healthy vs PDA Patients

![Dendrogram comparing bacterial taxa between NML and PDA groups.]

- a: Brevibacterium
- b: Brevibacteraeaceae
- c: Pedobacter
- d: Sphingobacteriaceae
- e: Sphingobacteriales
- f: Sphingobacteriia
- g: Chlamydiales
- h: Chlamydia
- i: Mogibacterium
- j: Oscillospira
- k: Methylobacteriacea
**Proteobacteria** is enriched in the human intra-pancreatic microbiome

**Question**

How does ablation of the microbiome affect the progression of pancreatic oncogenesis?
Germ Free Mice
National Gnotobiotic Rodent Research Center (UNC)

- KC mice steriley re-derived
- Maintained in barrier tight isolators
- Mice sacrificed at 3, 6, and 9 months
- Compared PDA progression to non-sterile KC mice

Germ Free KC Mice are Protected against PDA
Germ Free KC Mice are Protected against PDA

Pushalkar et al, Cancer Discovery 2018

Germ Free Mice are Protected against Pancreatic Oncogenesis (KC model)
Ablative Oral Antibiotic Regimen

- Vancomycin
- Ampicillin
- Neomycin
- Flagyl

Bacterial Ablation Protects Against PDA (Orthotopic KPC Model)
Question

Do hosts with PDA exhibit bacterial dysbiosis in the gut?

Stage-specific microbiome in PDA
Stage-specific microbiome in human PDA

The Gut Microbiome is Distinct in Murine PDA

*Phyla*
The Gut Microbiome is Distinct in Murine PDA

**LDA Analysis**
Question

Does direct gut repopulation with “Good” or “Bad” Microbes alter PDA Progression?

The Gut Microbiome in PDA mice promotes Pancreatic Oncogenesis
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Does single bacterial transfer accelerate PDA?

Single bacterial transfer accelerates PDA
Hypothesis

Modulating the gut microbiome affects anti-tumor immunity in PDA

M1 vs M2 macrophages

- M1 macrophage
  - IFN-γ
  - MHC II
  - TNFα
  - iNOS
  - IL-4
  - IL-13
  - IL-10
  - Glucocorticoids
  - CD206
  - IL-10
  - Arg1

- M2 macrophage
  - IL-1β
  - IL-6
  - IL-8
  - Growth factors (VEGF)
  - MMP
  - Promote Th2 response

- Anti tumor
  - TNFα
  - IL-12
  - Reactive nitrogen
  - Oxygen intermediates
  - Promote Th1 response
The Gut Microbiome in PDA mice promotes expansion of M2-like macrophages

Experimental Question

• Can gut bacterial extract directly affect macrophage polarization?
Extract from PDA gut microbiome induces suppressive macrophage phenotype

Experimental Question

• Does bacterial ablation enhance adaptive anti-tumor immunity?
Microbial ablation expands CD8+ T cell Infiltration in PDA

Microbial ablation results in T cell activation in PDA
Microbial ablation induces a cytotoxic CD8^+ T cell phenotype and Th1-polarization of CD4^+ T cells
Repopulation with PDA-associated microbiome reverses the immunogenicity associated with bacterial ablation

**Experimental Question**

- Is tumor protection associated with bacterial ablation in PDA T cell dependent?
Experimental Question

• Is tumor protection associated with bacterial ablation in PDA T cell dependent?
  - T cell adoptive transfer experiments
  - T cell depletion experiments

Adoptive Transfer of Tumor-Infiltrating T cells from antibiotic treated mice (but not control mice) is protective against PDA
T cell depletion reverses tumor-protection associated with bacterial ablation in PDA

Microbial ablation induces results in higher T cell expression of PD-1
Experimental Question

- Would antibiotic ablation of gut bacteria enable PD-1 targeted immunotherapy?

Microbial ablation enables efficacy for checkpoint-based immunotherapy in PDA
Microbial ablation enables efficacy for checkpoint-based immunotherapy in PDA

Experimental Question? Are microbiota responsible for macrophage-mediated adaptive immune collapse in PDA

Innate Immune cells

Macrophages

Activating Signals

Inhibitory Signals

Adaptive Immune cells

T cells

Tolerance

Cancer cells
**Experimental Strategy**

- T cell activation assays in the context of PDA-microbiome-entrained macrophages

### Extract from PDA gut microbiome induces a suppressive macrophage phenotype

- Unstimulated
- $\alpha$CD3/CD28
- $\alpha$CD3/CD28 + PDA Microbiome-entrained Mφ
- $\alpha$CD3/CD28 + Control Microbiome-entrained Mφ
Extract from PDA gut microbiome disables the capacity of macrophage to present antigen

**Question**

- How does the gut microbiome affect macrophage programming in PDA?
Microbes can induce pattern recognition receptor signaling in macrophages

TLR4 activation accelerates pancreatic cancer
**NLRP3 and TLR9 promote PDA**

- Daley et al, *JEM* 2017
- Zamirinis et al, *JEM* 2015

**NLRP3 signaling in TAMs promotes M2 differentiation**

- SSA
  - WT 33%
  - NLRP3−/− 18%
- SSA
  - CD206
  - WT 65%
  - NLRP3−/− 77%
NLRP3 signaling is necessary for TAMs to accelerate PDA

Dectin-1 deletion results in CD4^+ and CD8^+ T cell activation in PDA draining lymph nodes

Hypothesis

• The PDA microbiome induces crippling immune-suppression in PDA via diverse pattern recognition receptor (PRR) activation

Experimental plan

• Test extract from PDA vs control microbiomes on PRR reporter cell line activation
Extract from the PDA gut microbiome induces higher PRR signaling than normal gut microbiome

Upregulation of TLR signaling in KC vs KC germ-free pancreata
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The PDA microbiome promotes oncogenesis via TLR signaling

PDA microbiome-entrained macrophages are suppressive of T cell immunity in a TLR-dependent manner
Summary

• Micorbiota are >1000X more abundant in the cancerous pancreas.
• PDA is associated with gut bacterial dysbiosis.
• The PDA-associated microbiome, including *Bifido.* and *Fuso.* species, promote PDA progression.
• The PDA microbiome induces higher TLR2, TLR4, and TLR5 signaling in innate immune cells.
• Bacteria induce suppressive macrophage programming via TLR signaling. This leads to T cell suppression.
• Targeting the microbiome is protective against PDA in a T cell dependent manner and enables efficacy for PD1 based therapy.
Investigator-initiated neo-adjuvant Phase 1b “window” clinical trial in early PDA

- Resectable pancreatic cancer
- Initial 7 day lead-in with abx
- Initial 6 patients will be evaluated for DLT’s
- If < 2 DLT’s in the 1st 6 patients, then dose expansion in 14 add’l patients
- If >1 DLT in 1st 6 patients, then study paused and different dose/schedule considered
- Follow surgical resection, R0 resection rate and tumor regression grade will be evaluated by a dedicated pancreatic surgical pathologist and scored based on both the tumor regression

Intervention:
- Ciprofloxacin 500mg PO BID days 1-29
- Metronidazole 500mg PO TID days 1-29
- Pembrolizumab 200mg IV days 8 and 29
Do macrophages provide nutrient support to tumor cells?

Tumor-associated macrophages increase OCR in PDA cells
Tumor-associated macrophages increase OCR in PDA cells

Tumor-associated Macrophages Increase Tumor Cell Proliferation
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Metabolites from Tumor-Associated Macrophages Increase Tumor Cell Proliferation

TAMs Produce High Levels of Urea Cycle Intermediates

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Ornithine supplementation may accelerate tumor cell growth
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Tumors take up МΦ-derived Ornithine

Q: What do tumor cells use this ornithine for?

Tumors use МΦ-derived Ornithine to fuel urea cycle
Tumors use MΦ-derived Ornithine to fuel urea cycle

Q: Can ornithine impact other aspects of tumor metabolism?

Q: How does the increased urea cycle flux impact other aspects of tumor metabolism?
Ornithine supplemented tumors have higher AMPK signaling and possibly higher glucose intake.
Possible Directions and Questions to Explore

• i. What does this ornithine help the tumors do? Polyamines? Arginine-based compounds? ADP/ATP ratio to fuel glycolysis?

• ii. Where is the macrophage-derived ornithine coming from?

Arginine/Ornithine can be used to generate Polyamines

Prognostic Value of Polyamine Shunt Enzymes

**OAZ1**

- Expression cutoff: 1.05-6 FPKM
- 5-year survival log
- Event survival log
- Log-rank P-value

**AZIN2**

- Expression cutoff: 1.5 FPKM
- 5-year survival log
- Event survival log
- Log-rank P-value
Prognostic Value of Polyamine Shunt Enzymes

Can tumor metabolism influence МΦ phenotype?

Ornithine

Tumor

МΦ

?
Thank you!!!