Surgery Research Conference

Novel Inhibition Strategies for Pancreatic Cancer using Mice

To receive 1.0 credit for this session, text the SMS code: NARHAL to 414-206-1776. This code will expire in 5 days.

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Research Highlights
The American Surgical Association Surgical Leaders Fellowship Grant was awarded to Dr. Tracy Wang, who was selected by the ASA from the Society of Asian Academic Surgeons. Congratulations, Dr. Wang!

Tracy Wang, MD, MPH
Professor and Chief, Section of Endocrine Surgery, Division of Surgical Oncology
Congratulations
Erin Strong, MD, MBA, MPH!
Research Resident, General Surgery

2019-2020 Collaborative for Healthcare Delivery Science (CHDS) fellowship: The fellowship is one component of the educational arm of CHDS, which seeks to provide an instructional framework for trainees interested in projects that improve safety, quality, or patient experience, and that will improve patient outcomes for the same or lower costs.

- **Erin Strong**: The Rothman Index & physician utilization of early clinical warning systems (General Surgery)
- **David Vyles**: Penicillin Allergy and the Effect on the CHW Health System (Emergency Medicine)
Award Summary: to enable or augment research studies requiring additional resources to support the work, equipment purchases include lab equipment, scientific instruments, software purchases and specialty reagents. Priority will be given to projects that fit within the focus areas of the AHW funded award, *Improving Heart Health, Supporting Healthy Minds, and Dismantling Cancer*.

**Maximum Award:** $30,000  
**Award Period:** 18 months  
Application Due in REDCap: October 1st at 5pm  
Notification of Award: November 15th  
Project Start Date: December 2nd  
Contact: Lizzy Schneider

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Award Summary: The award provides financial support for formalized, faculty-defined projects over a concentrated period of time which benefit both the faculty member and MCW.

**Sponsor:** Faculty Affairs  
**Workshop Date:** Tuesday, November 5th  
**Time:** 8-9 am  
**Location:** HUB A1520  
**Application Due Date:** Friday, January 10th  
**Contact:** Kimara Ellefson, Associate VP Talent Strategy and Faculty Affairs
Surgical Oncology


General Surgery

Pediatric Congenital Cardiac Surgery
Extracorporeal Life Support and Increased Risk of Stroke with Carotid Cannulation. American Society for Artificial Internal Organs (ASAIO). (Woods, RK)

Pediatric Surgery


Next Month:

2017 We Care Award Updates:

Optimizing Cardiopulmonary Bypass to Support Cerebral and Somatic Perfusion During Aortic Arch Reconstruction

Viktor Hraska, MD, PhD
Professor and Chief
Pediatric Congenital Cardiac
Department of Surgery

October 9th, 2019
HRC 1210/1230/1250
5:00-6:00pm

Please note the
ROOM CHANGE
Novel Inhibition Strategies for Pancreatic Cancer using Mice

Clin Cancer Res; 18(16); 4266–76
Cancer TME

**Tumor vasculature**
- Bevacizumab (anti-VEGF-A)
- Sunitinib, Sorafinib (RTK inhibitors)
- Aflibercept (decoy receptor)

**Inflammatory pathways**
- Siltuximab (anti-IL-6)
- Ruxolitinib (JAK1/2 inhibitor)

**Repolarization and re-education**
- BLZ945, PLX3397 (anti-CSF-1R)
- CD40 mAb (agonist)

**Tumor stroma disruption**
- Nab-paclitaxel (Albumin-bound chemotherapeutic agent)

**Immunomodulation**
- PLX3397 (anti-CSF-1R, anti-KIT)
- Plerixafor (anti-CXCR4)
- S-265610 (anti-CXCR2)
- Trabectedin (chemotherapy)
- Gemcitabine, 5-FU (chemotherapy)
- Cisplatin (chemotherapy)

**Immune checkpoint blockade**
- Ipilimumab (anti-CTLA-4)
- Nivolumab (anti-PD1R)

**Key:**
- Tumor cell
- Dendritic cell
- B cell
- Extracellular matrix
- Fibroblast
- T cell
- Mesenchymal SC
- Basement membrane
- Macrophage
- Treg cell
- MDSC
- Blood or Lymphatic vessel

TRENDs in Cell Biology
Outline

A. Tools & Methods
   1. Cell Lines
   2. Mouse Models
   3. Histology & Molecular Biology
B. People - the TEAM and I
C. TGFβ Paradox: Compartment makes the difference
D. A New TGFβ family member & Target for PC: Activin
E. Therapy turned foe in a High Fat setting: PI3K Inhibition
F. Implications
Tools and Methods
Cell Lines

HPDE

HPDE-Kras cells

Stellate cells

MiaPaCa2

BxPC3

Panc
Mouse Models

Tools & Switches

1. a candidate gene - usually altered in human cancer
   mt Kras, mt p53, Smad4, p16 loss

2. a gene switch (promoter) that can target specific cell types
   Elastase (EL), Pdx1, p48

3. ability to genetically manipulate in order to insert the gene
   transgenesis, targeted manipulation,
Transgenesis: EL-Kras

5’ MT hss → 112 bp → 180 bp → 160 bp → 115 bp → 3’ MT hss

EL 1 2 3 4B hGH

mutation  human \(\text{KRAS}^{G12D}\)

polyA
ES Cell Manipulation

Gene Targeting Technology for Knockout/Knockin Mice

Mouse blastocyst → ES cells → Modified gene in targeting vector → Electroporation → Positive-negative selection → Inject to blastocyst → Implanted into foster mother → Chimeric mice
Mouse Pancreatic Lesions Resemble Human

PanINs

Cystic Neoplasms

human

mus

$\text{EL-KRAS}^\text{G12D}$

Cancer Res. 2003 May 1;63(9):2016-9
Mouse Pancreatic Lesions Resemble Human

PanINs

human

Pdx1-Cre/LSL-Kras (KC)
Cancer Cell. 2003 Dec;4(6):437-50

mus

Pdx1-Cre/LSL-Kras (KC)
Cancer Cell. 2005 May;7(5):469-83

PDAC

Pdx1-Cre/LSL-Kras/p53^{R172H} (KPC)
Cancer Cell. 2005 May;7(5):469-83
## Mouse vs. Human

### Cystic Neoplasms

<table>
<thead>
<tr>
<th>human</th>
<th>mus</th>
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<tbody>
<tr>
<td><img src="image1.png" alt="Image 1" /></td>
<td><img src="image2.png" alt="Image 2" /></td>
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### PanINs

<table>
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<td><img src="image3.png" alt="Image 3" /></td>
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</table>

*References*

- Cancer Cell. 2003 Dec;4(6):437-50
- Cancer Res. 2003 May 1;63(9):2016-9
- Pdx1-Cre/LSL-Kras
- EL-Kras
- Pdx1-Cre/LSL-Kras
A. Gross & Microscopic Pathology
   1. Tissue histology (pancreas, liver, GI tract, spleen)
   2. Cell culture (cytospin and/or grown on chamber slides)
   3. IHC/IF staining of proteins (biomarkers) & localization
   4. Lesion and stain scoring
B. Protein Quantitation: Western blot analyses
C. Cell mobility: scratch wound assay
People: The TEAM & I
The Purple PIMP
TGFβ Paradox: Compartment/Timing makes the difference
TGFβ Paradox

Common Alterations in PC

TGFβ Paradox

TGFβ & Kras in PC Subtypes

TGFβ Paradox

The Research Team Then

Danny Principe  Emman Mascariñas  Dr. David Bentrem

Brian DeCant  Dr. Morgan Barron  Kevin Adrian

Dr. Boris Pasche  Dr. Barbara Jung
What are the roles of TGFβ signals during the development of pancreatic neoplasms?

How do TGFβ and Kras signals interact and are there differences depending on cancer state?
Penetrance, Frequency, and Size of CPN lesions

EL-Kras/Tgfbr1\(^{+/-}\) have less lesions vs. EL-Kras

Cancer Res. 2009 Dec 15;69(24):9169-74
What we do know about TGFβ signals in PC

1. enforce immunosuppression to protect pancreatic tumor cells
2. promote EMT and the expansion of the TME
EL-Kras ± altered TGFβ signals
## TGFβ Paradox

<table>
<thead>
<tr>
<th>EL-Kras</th>
<th>EL-Kras/Tgfbr2</th>
<th>EL-Kras/Tgfbr1</th>
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</table>

**Tgfbr1⁺⁻ may increase T-cell Cytotoxicity**
TGFβ Paradox

TGFβ signals support Treg cell function
TGFβ Paradox

**EL-Kras**

**EL-Kras/Tgfbr1**

**PCNA**

**Granzyme B**

Tgfbr1+/− increases T-cell Cytotoxicity
Adoptive Transfer of TGFBR1-Deficient CTLs

Cancer Res. 2016 May 1;76(9):2525-39
TGFβ Paradox

Immune Interface Checkpoints

[Diagram showing TCR and MHC with various coinhibitory molecules such as CTLA-4, PD1, CD80, BTLA, TIM3, CD80/CD86, PD-L1/PD-L2, PD-L1, HVEM, GAL9]
Preclinical Assessment: TGFBR1 & PD-1 Inhibition

TGFβ Paradox

Body Weight (g)

KPC  +Invivomab (PD-1)  +Galunisertib (TGFBR1)

KPC - vehicle

KPC – Gal + Inviv
TGFβ Paradox

Preclinical Assessment: TGFBR1 & PD-1 Inhibition

Mol Cancer Ther. 2019 Mar;18(3):613-620
TGFβ Paradox
A New TGFβ family member & Target for PC: Activin
New Target: Activin

modified from *Front Mol Neurosci*. 2016 May 10;9:32. doi: 10.3389
New Target: Activin

The Research Team Now (sort of)

[Image of a group of people at a table]

Jonas
Georgi
Carolina
Karla
New Target: Activin

Building Acvr1a Mouse Models
Loss of Acvr1a in PC Mice improves survival

New Target: Activin
New Target: Activin

Loss of Alk2 in KPC mice delays metastasis

KPC

KPCA⁻/⁻

5 months of age
New Target: Activin

Expression of Acvr1a-CA increases migration
New Target: Activin

Expression of Acvr1a-CA in KC mice
New Target: Activin

Differences between Activin & TGFβ regarding AKT

- TGFβ signals - appear to work through pERK
- Activin signals - appear to work through pAKT
- Activation of AKT via phosphorylation is worth considering
- PI3K could be a reasonable Target
Another Target: PI3Kγ

PI3Kγ is overexpressed in human PC

Normal Patient 1 Patient 2

p110γ

Mean p110γ score

Normal PDAC

*
Another Target: PI3Kγ

Building PI3Kγ Mouse Models

KC x PI3Kγ CA → KC PI3KγCA
KC x PI3Kγ KO → KC PI3Kγ⁻⁻
Another Target: PI3Kγ

PI3Kγ is overexpressed in mouse PC

B6  KC  KPC

Mean p110γ score

p110γ
Another Target: PI3Kγ

Loss of PI3Kγ reduces pAKT & disease burden
High Fat Diet blocks effects of PI3Kγ loss

Another Target: PI3Kγ
Another Target: PI3Kγ

KC-PI3Kγ mice develop severe steatosis
Another Target: PI3Kγ

PI3Kγ associates with increased expression of PI3Kα & β

**p110α**

**p110β**

PNAS. 2019 Jul 16;116(29):14724-1473
Implications

• Where genes/oncogenes are altered matters: epithelial vs. mesenchymal
  • Non-epithelial TGFβ signals are more sinister by suppressing immune support
• New treatements are likely when considering novel targets
  • Activin and/or its receptors may serve as points of therapy for improved survival
• Combined therapies have potential benefits
  • TGFβ and Activin may work well in conjunction with PD-1/PD-L1 dual targeting
• Beware of Complications/Toxicity due to metabolic imbalance(s)
  • Fatty Liver Disease may indeed impact cancer therapies
Main Collaborators
Dr. Barbara Jung
Dr. HG Munshi (NU)
Dr. David Bentrem (NU)

EL-Kras/TGFβ Project
Dr. Boris Pasche (Wake-Forest)
Dr. Qinghua Zeng
Dr. Mike Pennison
Dr. Laurent Bartholin (INSERM)

Lab Investigators
Georgina Mancinelli
Karla Castellanos
Ronald McKinney
Dr. Carolina Perales-Torres
Dr. Steve Waters
Danny Principe
Andrew Diaz
Emman Mascarinas
Morgan Barron
Kevin Adrian
Akilesh Honosage
Brian DeCant
Dr. Michelle Schultz

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H-Foundation Bridge (NU)
Internal Support – DOM (UIC)
Be Bold . . . and Take the Cat by the Ears