Accelerating Therapeutic Discovery with Organoid and Mouse Models of Pancreatic Disease

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Collaborative Effort to Develop New Models of Pancreatic Cancer

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Derivation of Human Pancreatic Ductal Organoids

Normal Pancreas from Islet Transplantation Center

- Biopsy
- Digested excess exocrine
- Samples shipped on ice
- 2 - 4 hr digestion Collagenase TrypLE
- Plate normal cell mixture in Matrigel

72 hr later organoids begin to be visible

PDA Surgical Tumor Resection

- Tumor Resection
- Small normal specimen selected by Pathology
- Sample arrives ~4 - 5 hr after surgery
- 2 - 4 hr digestion Collagenase TrypLE
- Plate normal cell mixture in Matrigel

Primary or Metastasis Fine Needle Biopsy

- FNA biopsy arrives ~4 - 5 hr after the procedure
- Red blood cell lysis
- No digestion
- Plate cell mixture in Matrigel
Human Pancreatic Organoids Can Be Isolated From the Full Spectrum of Disease
High Success Rate of Organoid Generation

Samples sent overnight and processed the following day

Exclusions: non-PDA and shipping errors
Failures: Successful chemotherapy, Insufficient viable material, Failure to expand
Organoids Exhibit Expected Genetics

Confidential
Organoids Exhibit Expected Genetics: WES

Organoids
Primary

Primary Allele Frequencies (MAFs) of primary tissue and corresponding organoid culture reveals subclonal genetic alterations masked in the primary specimen.

MAF

Organoid

Primary

KRAS

p53
Orthotopic Transplantation of Human Organoids leads to stromal recruitment and collagen deposition: hT3

Host: NSG immuno-compromised mice
Using Models to Advance Pancreatitis and Pancreatic Cancer Research

Modeling patient response in human pancreatic cancer organoids

From Correlative to Causative? CA19-9 expression and Pancreatitis
Feasibility/Retrospective Study to Validate the Organoid Model

Organoid based patient profiling

Stage I  Stage II  Stage III  Stage IV
Resectable disease  Unresectable disease
Surgical specimen  Biopsy specimen
Germline DNA  Organoids  Tumor DNA
Genomics  Transcriptomic  Pharmacotyping

n = 50 successful cultures per arm
Organoid Pharmacotyping: 4 – 6 weeks

Drug concentration (M)

% viability

Log Drug

ALIVE

DEAD

Gemcitabine

Paclitaxel

SN-38

5-FU

Oxaliplatin

Complete Media

Matrigel

Organoids

Dissociate & Plate Single Cells

10% Matrigel Overlay

Organoids Reform

Drugs

Luminescence

Cellular ATP

hM1A

Drug viability

ALIVE

DEAD
Comparing Patient-Derived Organoid Response to Chemotherapeutics: AUC

AUC z-score

More resistant (non-responders)

More sensitive (responders)

Gemcitabine, Paclitaxel, SN-38, 5-FU, Oxaliplatin
Comparison of Patient and Organoid Therapeutic Responses

**hF2**
Stage 4 at diagnosis

3 year survival

Progression on FOLFOX, ABX

Sensitive to Gem, Iri, 5FU treatment
Comparison of Patient and Organoid Therapeutic Responses

**hF2**
Stage 4 at diagnosis
3 year survival
Progression on FOLFOX, ABX
Sensitive to Gem, Iri, 5FU treatment

**hF23**
Stage 3 at diagnosis
Gem/ABX 7 cycles with SD
CA19.9 decreased from 1691 to 589
Longitudinal Comparison of Patient and Organoid Responses


FOLFIRINOX | GEM/ABX
PDA
Diagnosis and resection
No sample collection

Lung Met biopsy
Diagnosis and resection
hM1A organoid

FOLFIRINOX | GEM/ABX
Disease progression
hM1E organoid
Death and autopsy
hM1F organoid

Gemcitabine
Paclitaxel

SN-38 5-FU Oxaliplatin

AUC z-score

Resistant
gemcitabine paclitaxel SN-38 5-FU oxaliplatin

Sensitive
Targeted Inhibitor Screens in Organoids

Everolimus is active in both hM1E and hM1F.
The Value of Precision Medicine for “Extraordinary” Responders

Patient died prior to getting most active chemotherapy agent

To maximize the potential benefit to the patient, pharmacotyping must be done rapidly

Should this be the basis for treatment?

Stage 4
5 weeks survival post diagnosis
One course of GEM/OXALI

To maximize the potential benefit to the patient, pharmacotyping must be done rapidly
Achieving a Clinically Relevant Time Frame for Organoid Testing in First Line is Challenging

Accelerated testing will require higher assay sensitivity and kinetic monitoring to account for:

- Culture heterogeneity
- Slower growth rate
- Low cell number
Modeling Patient Response Using Human Pancreatic Cancer Organoids: Summary

- Human pancreatic organoids can be isolated with high efficiency from the full spectrum of disease
- Anecdotal evidence that organoids accurately model patient response
  - Clinical trial underway
  - Identification of extraordinary responders
  - Longitudinal Modeling
- Organoid pharmacotyping can be performed within 4 – 6 weeks
  - Decreasing the latency of therapeutic testing
Using Models to Advance Pancreatitis and Pancreatic Cancer Research

Modeling patient response in human pancreatic cancer organoids

From Correlative to Causative? CA19-9 expression and Pancreatitis
CA19-9: Correlative or Causative?

- CA19-9 is elevated in gastrointestinal diseases
  - Used to follow treatment response in pancreatic cancer patients
  - ~30% of pancreatitis patients elevate CA19-9 in their blood

- CA19-9 (sialyl Lewis\(^a\)) is a glycan modification attached to many proteins
CA19-9: Correlative or Causative?

Health Individuals: disialyl Lewisα

Pancreatic Disease: sialyl Lewisα (CA19-9)

Mice
Making CA19-9 expression mouse models: CRE- and Dox- dependent

FB: Dox and rtTA dependent promoter driving FB enzymes
LSL: CRE-dependent expression of rtTA
Pdx: Mosaic expression of CRE in pancreas, duodenum, and bile duct
FB;LSL;PDX mice exhibit Pancreatitis upon CA19-9 expression.
FB;LSL;PDX mice exhibit Pancreatitis upon CA19-9 expression

The vast majority of the pancreas exhibits signs of pancreatitis (ADM, atrophy)

Pancreatic enzymes levels in the blood are elevated within 24 hours of Dox induction of CA19-9 expression
FB;LSL;PDX mice exhibit Pancreatitis upon CA19-9 expression

No changes to T- or B- cell numbers were observed at these time points

Like the Cerulein (CCK analog) model of acute pancreatitis, CA19-9 expression causes an influx of inflammatory monocytes within 72 hours
FB;LSL;PDX mice exhibit Pancreatitis upon CA19-9 expression

CA19-9 causes elevated levels of proliferation in the infiltrating immune cells and ductal compartment
Normal Organoids with Inducible CA19-9 Expression

GFP: Readout for expression of the enzymes required for CA19-9 expression
mKate: Readout for Cre-mediated recombination in the pancreas
Normal Organoids with Inducible CA19-9 Expression

CA19-9 expression correlates with activation of signaling pathways

Phospho-Protein Levels FB;LSL;PDX Organoids

Normalized Fold Change Relative to 0H

Hours of Dox:

CA19-9
pEGFR
pAKT
tAKT
pERK
tERK
Cofilin
Can CA19-9 be Used to Treat Pancreatitis?

Modeling CA19-9 targeting with Dox withdrawal

Using a CA19-9 blocking mAb mlgG

PILOT

CA19-9 mAb NS19-9 reduces level of pancreatic enzyme release in vivo
CA19-9 Plays a Role in Mediating Pancreatic Disease

- Expression of CA19-9 in the mouse pancreas causes rapid development of pancreatitis
  - Histologic hallmarks of pancreatitis
  - Elevation in pancreatic enzyme levels in the blood
  - Recruitment of immune cells
  - Induction of proliferation

- CA19-9 expression does not result in autoimmune reactions
  - Other organs show no sign of inflammation or fibrosis

- Future work will focus on the changes to signaling pathways in response to CA19-9 expression & therapeutic targeting using CA19-9
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Questions?