Mouse Modeling for Human Pancreatic Cancer
Gloria Su, Ph.D.
gs2157@columbia.edu, ICRC 10-04
Professor
Departments of Pathology & Cell Biology
Otolaryngology/Head & Neck Surgery
Columbia University Medical Center

Modeling Human Pancreatic Cancer
- Pancreatic cancer etiology
  - Mouse models for human PC
    - xenograft
    - carcinogen-induced
    - genetically engineered mouse models (GEMMs)
    - 3-D organoids
- Research Trends/Looking ahead

Our Success

Cancer History
- DNA structure was proposed by Drs. Francis Crick and James Watson in 1953.
- "Two-hit hypothesis": multi-mutation theory on cancer-first published by Carl Nordling in 1953; popularized by Alfred Knudson in 1971 and also known as "Knudson’s Hypothesis".
- 1971, President Richard Nixon declared war on cancer.
- 1976, Drs. J. Michael Bishop and Harold Varmus discovered the first oncogene (Nobel Prize winners, 1989).
- 1986, Dr. Robert Weinberg discovered the first human tumor-suppressor gene (TP53).
- 1994, Dr. Mark Skolnick linked TSG to familial breast and ovarian cancer, confirmed by Dr. Mary-Claire King the same year.
- 1984, oncogenic KRAS was discovered as an oncogene in lung cancer, and subsequently in PC in 1988.
- 1996, DPC4/HMAD4/SMAD4 was discovered by Dr. Scott Kern.

Pancreatic Cancer
- Pancreatic cancer is relatively rare- the eleventh most common cancer in respect to incidence in the USA.
- The 3rd leading cause of cancer death (only behind lung and colorectal cancers) due to poor prognosis in the USA- five-year survival rate is ~8%.
- The highest rates of pancreatic cancer tend to occur in the developed countries.
- The risk factors includes aging, current smoking (OR: 2.20), heavy drinking (>3 drinks/day), obesity (body mass index >30kg/m²), diabetes (>3 years), family history of PC (OR:1.6), non-O ABO genotype, certain SNP alleles, chronic inflammation.
- Pancreatic cancer is a result of accumulative genetic mutations.
Pancreatic Cancer

- >95% are derived from exocrine cells, including pancreatic ductal adenocarcinoma (PDAC), medullary carcinoma, acinar cell carcinoma, pancreatoblastoma, etc.
- The majority of human pancreatic cancer are presented as PDAC that progress from progress from three different precancerous lesions called pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasms (IPMNs), and mucinous cystic neoplasms (MCNs).
- <5% are from endocrine lineage- pancreatic neuroendocrine tumors (PNET)

Pancreas

- The pancreas is made up of three cell lineages-islet, acinar, and ductal epithelial cells.

Pancreatic Cancer Action Network, Incidence Report 2012

Pancreatic Cancer Action Network, Trends Report 2017

Increasing Trend in PC Incidence Worldwide

Pancreas

- Pancreatic Cancer Action Network, Incidence Report 2012
- Pancreatic Cancer Action Network, Trends Report 2017
- Klapman & Malafa, Cancer Control 2008
PanIN (pancreatic intraepithelial neoplasia)  
- Progresses to pancreatic ductal adenocarcinoma (PDA).
- Microscopic papillary or flat noninvasive.
- Arise from intralobular ducts.
- Columnar-to-cuboidal cells with varying amounts of mucin and degrees of cytologic and architectural atypia.
- Ducts less than 5 mm in diameter.
- Five-year survival following resection is less than 20%.

IPMN (intraductal papillary mucinous neoplasm)  
- Progresses to non-invasive or invasive carcinoma.
- Grossly visible, noninvasive, mucin-producing predominantly papillary or rarely flat, epithelial neoplasm.
- Arising from the main pancreatic duct or branch ducts.
- Lesions greater than 1 cm in diameter.
- Five-year survival following resection of IPMN with invasive cancer (43%) or without invasive cancer (77%).

PanIN vs. IPMN

The Importance of Cancer Genetics Profiling
- Early Detection-High-risk patients (familial and sporadic) and follow-up/recurrence.
- Biomarkers-Prognosis, diagnosis, staging.
- Therapeutic targets
- Chemoprevention
- Animal modeling

Genetics of Cancer
- Tumor suppressor genes—normally function to restrain cell proliferation, and loss of their activity may lead to unrestrained cell growth (broken brake pedal).
- Oncogene—encode for protein which, when overexpressed or activated by mutation, possess transforming properties (gas pedal stuck in the “on” position).
- DNA Mismatch Repair Gene—check the fidelity of DNA replication. When inactivated, errors which normally occur during DNA replication are not corrected (Drunk mechanic).

Global genomic analyses of pancreatic cancer
- 63 alterations can be defined by 12 core signaling pathways.

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<th>Gene</th>
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<th>Frequency (%)</th>
<th>Mutation Origin</th>
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<td>SMAD4</td>
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<td>som.</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>3p14.2</td>
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<td>som.</td>
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Su et al. Curr Opinion in Gastro, 2000
Genomic analyses and subtypes of pancreatic cancer

- 456 pancreatic ductal adenocarcinomas identified 32 recurrently mutated genes that aggregate into 10 pathways: KRAS, TGF-β, WNT, NOTCH, ROBO/SLIT signalling, G1/S transition, SWI-SNF, chromatin modification, DNA repair and RNA processing.

Majority of mutations occur before metastasis

- Sequencing the genomes of 7 pancreatic metastases.
- A total of 426 somatic mutations in 388 different genes were identified among ~221 millions base pairs sequenced, corresponding to an average of 61 mutations per index metastatic lesion (range 41–77).
- Two categories of mutations were: 'founder' mutations (mutations present in all samples from a given patient; mean of 64%, range 48–83% of all mutations per patient). All other mutations were characterized as 'progressor' mutations.
- Parental, non-metastatic clones from primary tumors give rise to distal metastatic clonal populations.
- Within individual patients, a large majority of driver gene mutations are common to all metastasis- minimal functional gene heterogeneity among untreated metastasis.

Late diagnosis, not its intrinsic aggressiveness, causes high mortality in PC patients?

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Pancreatic cancer metastasis is regulated majorly by epigenetic events

- Three subtypes: classical, quasi-mesenchymal, and microsatellite-stable.
- Two tumor subtypes: classical and basal-like.
- Four subtypes: (1) squamous; (2) aberrant differentiation; (3) adenocarcinoma (ADEX); (4) immunogenic.

Expression analyses and subtypes of pancreatic cancer

- Four subtypes: (1) squamous; (2) aberrant differentiation; (3) adenocarcinoma (ADEX); (4) immunogenic.
Hurdles in PC Patient Care

- Low incidence.
- Most of PC patients die within 6 months of diagnosis.
- Only 15-20% of PC are surgically resectable.
- Resected tumors have lots of normal stromal tissue contamination.
- Early PanIN samples are rare.
- Complexity of mutations
- Desmoplasia complication
- Metastasis in most patients

The Needs in PC Patient Care

- Early Detection—High-risk patients (familial and sporadic) and follow-up/recurrence.
- Biomarkers—Prognosis, diagnosis, staging, predicting treatment responses.
- Therapeutic targets
- Chemoprevention
- Animal modeling

Mouse Modeling for Human Pancreatic Cancer

- A model that recapitulates its human counterpart in tumorigenesis (in both histological progression and genetic mutations).
- A model that allows spontaneous tumor development and yet with predictable time line.
- A model that has an intact microenvironment and yet allows metastasis.

Generating Models for PC

- Xenografts (human PC cells into mice)
  - Subcutaneous
  - Orthotopic
  - Patient-derived xenografts
  - Humanized mouse
- Carcinogen administration
  - BOP into hamsters
  - DMBA into mice & rats
  - Azaserine into rats
- Genetic Engineering
  - Oncogenes: mutant Kras, TGFalpha, SHH
  - TSG: p16, Smad4, p53, TGFbRII, Stk11, etc.
- 3-D organoids

Xenograft Mouse Models

Subcutaneous Xenograft

Implantation of PC from cell lines or resected tissue.

Cancer cells injected subcutaneously into immuno-compromised mice (nude or SCID)

Generate mice with pancreatic cancer under the skin

Orthotopic: Implantation into the pancreas

Orthotopic: Implantation into the pancreas

Tsuji et al, J Pancreas 2006; 7:193-9

Patient-derived xenografts

Tentler, J. J. et al. (2012) Patient-derived tumour xenografts as models for oncology drug development

Humanized Mice

- Mouse models with humanized immune systems.
- CD34+ humanized mice - models engrafted with cord blood-derived hematopoietic stem cells with robust T-cell maturation and T-cell dependent inflammatory response.
- PBMC humanized mice - models engrafted with adult peripheral blood mononuclear cells and enable short-term studies requiring a strong effector and memory T cell and NK cell functions.

Humanized Mice

Brehm et al, Current Opinion in Immunology 2013.
Hahn et al, Frontiers in Immunology, 2015

Morton et al, Cancer Research 2016
Xenofrat Mouse Models for PC

- Xenographs (human PC cells into mice)
  - Subcutaneous
    - Pros: Easy & cheap, short-term, high penetrance, easy to quantify tumor burden.
    - Cons: No metastasis, no PanIN, lacks intact TME.
  - Orthotopic
    - Pros: Metastasis & cheap, short-term, PanIN in some, high penetrance, better mimicking human PDAC histologically.
    - Cons: Labor intensive, PanIN in some, more mouse-to-mouse variability, more difficult to quantify tumor burden, lacks intact TME.
  - Patient-derived xenographs
    - Pros: Some as other xenographs plus the potential for personalized medicine.
    - Cons: Same as other xenographs
  - Humanized Mice
    - Pros: Humanized human system, personalized medicine.
    - Cons: Expensive and labor intensive, some limitations on immune cell diversity.

Carcinogen induced

- Carcinogen administration
  - Pros: Easy & cheap, PanIN in some models, simulate environmental assaults, intact TME.
  - Cons: Unknown genetic profile, difficult to monitor progression, few carcinogens have been studied in mice.


Genetically-Engineered Mouse Models

- Strategies
  - Transgenics
  - Knock-in
  - Knock-out
- Targeted genes
  - Oncogenes: mutant Kras, TGFalpha, SHH, GNAS
  - TSG: p16, SMAD4, p53, TGFbRII, STK11, etc.
- Targeted cell types
  - Pancreatic progenitor cells
  - Acinar cells
  - Ductal epithelial cells
  - Centroacinar cells

- Pros & Cons
  - Pros: Best mimicking human PC at genetic & histologic levels, intact TME, PanIN development, allows pathway analyses, progression to PDA and metastasis, early detection/biomarker discovery.
  - Cons: Expensive, time-consuming, labor-intensive, requires extensive knowledge on gene targeting, may have limited tumor complexity, may harbor secondary (not engineered) mutations, not suitable for personalized medicine.

Mutational Profile of Pancreatic ductal adenocarcinoma (PDA)

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<tr>
<th>Gene</th>
<th>Geno location (%)</th>
<th>Mutation origin</th>
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<tr>
<td>GNAS</td>
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<tr>
<td>MUC3B</td>
<td>4q24</td>
<td>som.</td>
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Tumor Suppression/Genome Maintenance Genes

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<tr>
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<tr>
<td>p53</td>
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<tr>
<td>TGFbRIV</td>
<td>16q12</td>
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<tr>
<td>APC</td>
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<tr>
<td>TGFbRII</td>
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<td>STK11</td>
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<td>MSH6</td>
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<td>MSH3</td>
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<tr>
<td>MSH4</td>
<td>2p12</td>
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How to target pancreatic ductal epithelium?
Existing Mouse Models for Pancreatic Cancer

Hruban et al, Can Res 2006; 66:95-106

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<td>KRAS</td>
<td>12p13</td>
<td>61</td>
<td>same</td>
</tr>
<tr>
<td>P16</td>
<td>11q13</td>
<td>16-20</td>
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</tr>
<tr>
<td>P48</td>
<td>6q24</td>
<td>36</td>
<td>same</td>
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The first mouse model that develops PanINs that simulate human precursor lesions.
- It utilizes both knock-in and conditional activation technologies.
- Conditional activation of KrasG12D at physiological level.
- The phenotypes of LSL-KrasG12D; Pdx1-Cre and LSL-KrasG12D; p48-Cre are very similar.

Pdx1-Cre; LSL-KrasG12D; p53R172H/+ mice (KPC) have accelerated tumor progression, but the same histology-PanIN to PDA

(Hingorani et al, Cancer Cell 2005)

Mouse Model #1-PanIN/Pancreatic Ductal Adenocarcinoma
PKP GEMM-

p16lox/lox; LSL-KrasG12D; Pdx1-Cre mice

Bardessy et. al., Can Dev 2006; Izeradjene et al, Cancer Cell 2007
pf6 inactivation works synergistically with oncogenic Kras (PKP mice) in promoting pancreatic tumorigenesis

Mouse Modeling for Human Pancreatic Cancer

- A model that recapitulates its human counterpart in tumorigenesis (in both histological progression and genetic mutations).
- A model that allows spontaneous tumor development and yet with predictable time line.
- A model that has an intact microenvironment and yet allows metastasis.

PKP mice developed PDAC and frequent metastasis

- PDAC is the major histologic presentation.
- Frequency of metastasis increased with age: 44% in mice younger than 4 months and 49% in those older than 4 months.
- Metastasis is observed in all non-thriving mice (n=14) and involves liver, LN, stomach, lung, testis, spleen, etc.

The histologic presentations of the KrasG12D oncomice differed for each tumor-suppressor gene

Activin signaling in pancreatic tumorigenesis

- We have previously reported sporadic biallelic inactivation of the Activin receptor type 1B (ACVR1B) gene in human pancreatic ductal adenocarcinoma (PDA) (Su et al, PNAS 2001).
- Activin signaling belongs to the TGF-β superfamily. Acvr1b<sup>−/−</sup> mice died at gastrulation. To investigate the significance of ACVR1B in cancer development, a conditional Acvr1b knockout mouse line was generated to interrogate its potential role as a tumor-suppressor gene in vivo.
Inflammation and ADM were observed in the pancreata of Acvr1b<sup>−/−</sup>;Pdx1-Cre mice

Acvr1b<sup>−/−</sup>; LSL-Kras<sup>G12D</sup>; Pdx1-Cre (AKP) mice exhibited shortened survival and IPMN histologic phenotype

The inactivation of Acvr1b favors the expansion and progression of IPMNs to PDA in AKP mice

The histologic presentations of the Kras<sup>G12D</sup> oncomice differed for each tumor-suppressor gene

What is the cell of origin for IPMN?
2D and 3-D cultures

Acinar to ductal metaplasia culture-Means et al, Development 2006; Qiu et al, Gastroenterology 2015

Primary pancreatic duct epithelial cell cultures-Agbnou et al, Cancer Res 2006

3-D Organoids

Boj et al, Cell 2015

Research Trends

Early Detection
- Exosomes
- Circulating tumor DNA (ctDNA)
- Circulating tumor cells (CTCs)

Combination therapies
- FOLFIRINOX
- Nab-paclitaxel (Abraxane) plus Gemzar
- Other target therapies (angiogenesis, metabolism, KRAS & other oncogenes, immunotherapies, autophagy, etc)
- Immunotherapy/vaccine

Metastasis
- Tumorigenesis
- Clonal expansion/tumor heterogeneity
- Treatment options

The Needs in PC Patient Care

Early Detection-High-risk patients (familial and sporadic) and follow-up/recurrence.

Biomarkers- Prognosis, diagnosis, staging, predicting treatment responses.

Therapeutic targets

Chemoprevention

Animal modeling

Pro & Con
- Pro: Allows personalized medicine, recapitulate PanIN and PDA histology and genetics, bypassing the question of cell origin, can be co-cultured with fibroblasts and immune cells for in vitro studies.
- Con: Lacks TME (if xenografted); require transforming growth factor β (TGF-β) pathway inhibitors (A83-01 and Noggin), R-Spondin1 and Wnt-3a-conditioned media, EGF, and PGE2 for propagation; potential issue of cell origin.

Research Trends

- Cell origin and early tumorigenesis
  - PanIN vs. IPMN
  - Ductal vs. Acinar
  - Epigenetic regulation in tumor initiation

- TME
  - Fibroblasts
  - Immune cells
  - Chemokines and cytokines

- Epigenetic regulations
  - Early tumorigenesis
  - Tumor subtypes
  - Metastasis
The Needs in PC Patient Care

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<th>Xenograft</th>
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<td>Personalized Medicine, predicting treatment responses</td>
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Early detection
Biomarker discovery– prognosis, follow-up/recurrence
Therapeutic targets discovery testing
Chemoprevention
Immunotherapy
Personalized Medicine- predicting treatment responses

R01 success rate was above 20% before 2005 and has been less than 10% in recent years

No relief in sight

2019 Gigi Shaw Arledge Conference on Pancreatic Disease
Monday, October 7, 2019

Vivian and Seymour Milstein Family Heart Center
Columbia University Irving Medical Center

LOCATION:
New York Presbyterian/Columbia University Irving Medical Center
170 Fort Washington Avenue, 1st Floor, New York, NY

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www.columbiaSurgeryCMF.org

For additional information, please contact Annmarie Tartman:
art2004@cumc.columbia.edu