Mouse Modeling for Human Pancreatic Cancer

# Wage Hope
November is Pancreatic Cancer Awareness month

ABOUT PancreATIC CANCER
Pancreatic cancer is the 3rd leading cause of cancer death in the United States.
Pancreatic cancer has the lowest 5-year survival rate of any cancer.
ONLY 8% of all pancreatic cancers are curable.

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 (TSG).
- 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/HRM4/SMAD4 was discovered by Dr. Scott Kern.

Cancer Statistics 2017 (Siegel et al, CA Cancer J CLIN 2017)
- A total of 1,688,780 new cancer cases and 600,920 deaths from cancer are projected to occur in the United States in 2017.
- Over the past decade of available data, the overall cancer incidence rate (2004-2013) was stable in women and declined by approximately 2% annually in men, while the cancer death rates (2005-2014) declined by about 1.5% annually in both men and women.
- For all sites combined, the cancer incidence rate is 2% higher in men than in women, while the cancer death rate is 49% higher. However, sex disparities vary by cancer type.
- From 1991 to 2014, the overall cancer death rate dropped 25%, translating to approximately 2,143,200 fewer cancer deaths than would have been expected.
- Although the cancer death rate was 15% higher in blacks than in whites in 2014, increasing access to care as a result of the Patient Protection and Affordable Care Act may expedite the narrowing racial gap; from 2010 to 2015, the proportion of blacks who were uninsured halved, from 21% to 11%, as it did for Hispanics (17% to 9%).

Epidemiology of Pancreatic Cancer
- Pancreatic cancer is relatively rare (the eleventh most common cancer in respect to incidence).
- The highest rates of pancreatic cancer tend to occur in the developed countries.
- The risk factors include 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 genetic disease.
The pancreas is made up of three cell lineages—insulin, acinar, and ductal epithelial cells.

Pancreatic Cancer
- >95% are derived from exocrine cells, including pancreatic ductal adenocarcinoma (PDAC), medullary carcinoma, acinar cell carcinoma, pancreatic blastomas, etc.
- The majority of human pancreatic cancers are presented as advanced lesions containing one or more of the following precancerous lesions called pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasms (IPMN), and mucinous cystic neoplasms (MCN).

PanIN (pancreatic intraepithelial neoplasia), IPMN (intraductal papillary mucinous neoplasms), and MCN (mucinous cystic neoplasms) are precancerous lesions of PDAC (pancreatic ductal adenocarcinoma).

PanIN (pancreatic intraepithelial neoplasia) vs. IPMN
- PanIN (progressive to pancreatic ductal adenocarcinoma (PDAC), microscopically papillary or flat, noninvasive, arising 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, 5-year survival following resection is less than 25%.

IPMN (intraductal papillary mucinous neoplasm) (progressive to noninvasive 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, 5-year survival following resection of IPMN with invasive cancer (43%) or without invasive cancer (77%)).

Invasive Cancer
- If a tumor is found to be malignant, its extent or spread is measured by a process called staging. The stages of pancreatic cancer are:
  - Stage I: Very small tumors limited to the pancreas (12-14%)
  - Stage II: Larger tumors localized to the pancreas (5-7%)
  - Stage III: The cancer has spread to the lymph nodes, although not necessarily to distant organs (3%)
  - Stage IV: The cancer has metastasized to the colon, spleen, stomach, or more distant organs such as the lungs or liver (1%)

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 (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).

Mutational Profile of Pancreatic ductal adenocarcinoma (PDA)

Global genomic analysis of pancreatic cancer (Jones et al, Science 2008)
- 24 pancreatic cancer, 33,219 transcripts, 20,661 genes, ~10^6 SNP-pancreatic cancer harbors 63 alterations on average, majority are point mutations.
Global genomic analyses of pancreatic cancer
- 63 alterations can be defined by 12 core signaling pathways.
- KRAS, TP53, SMAD4, CDKN2A, ARID1A, ROBO2 were confirmed and candidate drivers E2F3 and PDE6H were identified adding chromatin modification, nucleosome remodeling, and stem guidance signaling.

Genomic analyses and subtypes of pancreatic cancer
(Bailey et al, Nature 2016)
- 436 pancreatic ductal adenocarcinomas identified 32 recurrently mutated genes that aggregate into 10 pathways: KRAS, TGF-β, WNT, NOTCH, ROBO/SLIT signaling, G1/S transition, HRD-SNP, chromatin modification, DNA repair and RNA processing.

Expression analyses and subtypes of pancreatic cancer
(Bailey et al, Nature 2016; Yachida et al, Oncogene 2013)
- Three subtypes: classical, quasi-mesenchymal, and exocrine-like.

Majority of mutations occur before metastasis
- Sequencing the genomes of 7 pancreatic metastases.
- A total of 575 somatic mutations in 388 different genes were identified among 221 million base pairs sequenced, corresponding to an average of 6 mutations per index metastatic lesion (range 4–77).
- 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.
- Founder, non-metastatic clones from primary tumors give rise to distal metastatic clonal populations.

Late diagnosis, not its intrinsic aggressiveness, causes high mortality in PC patients
(Yachida et al, Nature 2010; Yachida et al, Oncogene 2013)
- Using Ki-67 as a marker to calculate cell doubling time, as well as the accumulation of passenger mutations to estimate number of passages, it was deduced that it takes at least a decade from the initiating mutation to the birth of a parental non-metastatic clone.
- At least five more years are required for the acquisition of metastatic ability and patients die an average of two years thereafter.

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 PanN 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

**Subcutaneous Xenograft**
- Implantation of PC from cell lines or resected tissue.

**Orthotopic: Implantation into the pancreas**

**Patient-derived xenografts**
- Tendler, 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

- Xeno grafts (human PC cells into mice)
  - Pros: Easy & cheap, short-term, high penetrance, easy to quantify tumor burden.
  - Cons: No microenvironment, PanIN, intact TME.
- Orthotopic
  - Pros: Metastasis, 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, intact TME.
- Patient-derived xenografts
  - Pros: Same as other xenografts plus the potential for personalized medicine.
  - Cons: Same as other xenografts.

Patient-derived xenografts

- Pro: Same as other xenografts plus the potential for personalized medicine.
- Con: Expensive and labor intensive.

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
  - Transgenic
  - 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 & Con
  - Pros: Best mimicking human PC at genetic & histologic levels, intact TME, PanIN development, allows pathway analysis, 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, may not be suitable for personalized medicine.

Mutational Profile of Pancreatic ductal adenocarcinoma (PDA)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>p16</td>
<td>10%</td>
<td>Allele</td>
</tr>
<tr>
<td>SMAD4</td>
<td>5%</td>
<td>Allele</td>
</tr>
<tr>
<td>TGFbRII</td>
<td>3%</td>
<td>Allele</td>
</tr>
<tr>
<td>STK11</td>
<td>2%</td>
<td>Allele</td>
</tr>
<tr>
<td>Kras</td>
<td>1%</td>
<td>Allele</td>
</tr>
<tr>
<td>TGFalpha</td>
<td>1%</td>
<td>Allele</td>
</tr>
</tbody>
</table>

How to target pancreatic ductal epithelium?

Existing Mouse Models for Pancreatic Cancer

- Humanized Mice
  - Pros: Humanized human system, personalized medicine.
  - Cons: Expensive and labor intensive.
- Carcinogen induced
  - 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.

Nishibori et al. Can Rev 2006; 06-05-016
The first mouse model that develops PanINs that simulate human precursor lesions.

- It utilizes both knock-in and conditional activation technologies.
- Conditional activation at physiological level.

The phenotypes of LSL-KrasG12D; Pdx1-Cre and LSL-KrasG12D; p48-Cre are very similar.

Mutational Profile of Pancreatic ductal adenocarcinoma (PDA)

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

Pdx1-Cre;LSL-KrasG12D;smad4-/- mice preferentially develop mucinous cystic lesions in the pancreases (MCN vs IPMN)

p16 inactivation works synergistically with oncogenic Kras in promoting pancreatic tumorigenesis

Medium survival of p16flox/flox; LSL-KrasG12D; Pdx1-Cre (PKP) mice is 25.5 weeks, which is considerably shorter than the LSL-KRASG12D; Pdx1-Cre (Kras) mice (~15 months).

PKP mice developed PDAC and frequent metastasis

- PDAC is the major histologic presentation.
- Frequency of metastasis increased with age: 46% 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

2D and 3-D cultures

Primary pancreatic duct epithelial cell cultures-Aghamir et al. Cancer Res 2006

3-D Organoids

Boj et al. Cell 2015
Pro & Con

- Pro: Allows personalized medicine, recapitulate PanIN and PDA histology and genetics, bypassing the question of cell origin.

- Con: Lacks TME (if xenografted); require transforming growth factor β (TGF-β) pathway inhibitors (A83-01 and Noggin), R-Spondin1 and Wnt3a-conditioned media, EGF, and PGE2 for propagation, potential issue of cell origin.

Boj et al., Cell 2015