Mouse Modeling for Human Pancreatic Cancer

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Modeling Human Pancreatic Cancer

- History of cancer
- Pancreatic cancer etiology
- Mouse models for human PC-xenograft, carcinogen-induced, genetically engineered mouse models (GEMMs), 3-D organoids
- Lessons from GEMMs

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 rate (2005-2014) declined by about 1.5% annually in both men and women.
- For all sites combined, the cancer incidence rate is 20% higher in men than in women, while the cancer death rate is 40% 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 (31% to 16%).

Epidemiology of Pancreatic Cancer

- 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.
- 1996, DPC4/HMAD4/SMAD4 was discovered by Dr. Scott Kern.

Pancreatic cancer is relatively rare (the eleventh most common cancer in respect to incidence). >53,670 Americans will be diagnosed with pancreatic cancer in 2017.

The 3rd leading cause of cancer death due to poor prognosis (~ 43,090 deaths estimated for 2017)- a five-year survival rate is ~8% (2005-2011).

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 (~5 years), family history of PC (OR:1.6), non-O ABO genotype, certain SNP alleles, chronic inflammation.

Pancreatic cancer is a genetic disease.
Siegel et al. CA Can J Clin 2017

Increasing Trend in PC Incidence Worldwide

Pancreas

Pancreatic Cancer

- >95% are derived from exocrine cells, including PDAC, medullary carcinoma, acinar cell carcinoma, pancreatoblastoma, etc.
- The majority of human pancreatic cancer are presented as pancreatic ductal adenocarcinoma (PDAC) that progress from PanIN (pancreatic intraepithelial neoplasm) lesions.
- <5% are pancreatic neuroendocrine tumors (PNET)

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

Pancreas

- The pancreas is made up of three cell lineages-islet, acinar, and ductal epithelial cells.
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%)
- GNAS, RNF43, STK11, PIK3CA

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

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 lung or liver (7%).

Global genomic analysis of pancreatic cancer (Jones et al, Science 2008)
- 24 pancreatic cancer, 23,219 transcripts, 20,661 genes, ~10^6 SNP-pancreatic cancer harbors 63 alterations on average, majority are point mutations.
Global genomic analyses of pancreatic cancer
(Jones et al., Science 2008; Biankin et al., Nature 2012; Waddell et al., Nature 2015)
- 63 alterations can be defined by 12 core signaling pathways.
  - KRAS, TP53, SMAD4, CDKN2A, ARID1A, ROBO2 were confirmed and candidate drivers KDM6A and PREX2 were identified—adding chromatin modification, nucleosome remodeling, and axon guidance signaling.

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

Expression analyses and subtypes of pancreatic cancer
(Collisson et al., Nature Med 2011; Moffitt et al., Nature Genetics 2015; Bailey et al., Nature 2016)
- Three subtypes: classical, quasi-mesenchymal, and exocrine-like.
- Two tumor subtypes: classical and basal-like.
- Four subtypes: (1) squamous; (2) aberrantly differentiated endocrine exocrine (ADEX); (3) pancreatic progenitor; and (4) immunogenic that correlate with histopathological characteristics and prognosis.

Majority of mutations occur before metastasis
(Yachida et al., Nature 2010, Yachida et al., Oncogene 2013)
- Sequencing the genomes of 7 pancreatic metastases.
- A total of 426 somatic mutations in 38 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.

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.
PanINgram

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%).

Desmoplasia—Friend or foe?

- Current model: The stroma is protective and is exerted at the PanIN stage (Ozdemir et al, Cancer Cell 2014, Rhim et al, Cancer Cell 2014)

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.
- 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
- Carcinogen administration
  - BOP into hamsters
  - DMBA into mice & rats
  - Azaserine into rats
- Genetic Engineering
  - Oncogenes: mutant Kras, TGFalpha, SHH
  - TSG: p16, Smad4, p53, TGFβRII, Stk11, etc.
- 3-D organoids

Orthotopic: Implantation into the pancreas

Orthotopic: Implantation into the pancreas

Patient-derived xenografts

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

Subcutaneous Xenograft

Implantation of PC from cell lines or resected tissue.

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

Generate mice with pancreatic cancer under the skin

Orthotopic: Implantation into the pancreas


Orthotopic: Implantation into the pancreas


Orthotopic: Implantation into the pancreas

Carcinogen induced

**Mouse Models for PC**

- **Xenografts (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 xenografts**
    - Pros: Same as other xenografts plus the potential for personalized medicine.
    - Cons: Same as other xenografts

- **Carcinogen administration**
  - BOP into hamsters, DMBA into mice & rats, Azaserine into rats
    - 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.

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**Genetically-Engineered Mouse Models**

- **Strategies**
  - Transgenesis
  - 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

- **Pro & Con**
  - Pros: Best mimicking human PC at genetic & histologic levels, intact TME, PanIN development, allows pathway analysis, progression to PDA and metastasis.
  - Cons: Expensive, time-consuming, labor-intensive, requires extensive knowledge on gene targeting, may have limited tumor complexity, may harbor secondary (not engineered) mutations.

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**Mutational Profile of Pancreatic ductal adenocarcinoma (PDA)**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Cislocation</th>
<th>Frequency (%)</th>
<th>Tumor suppressor/Gene Maintenance Genes</th>
</tr>
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<tbody>
<tr>
<td>ARID1B</td>
<td>1q21</td>
<td>5%</td>
<td>p16</td>
</tr>
<tr>
<td>MSH3</td>
<td>6q24</td>
<td>10%</td>
<td>p14</td>
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Tumor Suppressors/Gene Maintenance Genes:

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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_

**LSL-Kras\textsuperscript{G12D}; Pdx1-Cre and LSL-Kras\textsuperscript{G12D}; p48-Cre oncomice**
(Hingorani et al, Cancer Cell 2003)

- The first mouse model that develops PanINs that simulate human precursor lesions.
- It utilizes both knock-in and conditional activation technologies.
- Conditional activation of \textsuperscript{G12D} at physiological level.
- The phenotypes of \textit{LSL-Kras\textsuperscript{G12D}; Pdx1-Cre} and \textit{LSL-Kras\textsuperscript{G12D}; p48-Cre} are very similar.

**Mutational Profile of Pancreatic ductal adenocarcinoma (PDA)**

_Bardessy et. al., Can Dev 2006; Izeradjene et al, Cancer Cell 2007_

_Pdx1-Cre;LSL-Kras\textsuperscript{G12D}; p53\textsuperscript{R172H/0} mice have accelerated tumor progression, but the same histology-PanIN to PDA_

(Hingorani et al, Cancer Cell 2005)

_Pdx1-Cre; LSL-Kras\textsuperscript{G12D}; Smad4\textsuperscript{-/} mice preferentially develop mucinous cystic lesions in the pancreases (MCN vs IPMN)_

Bardessy et. al., Can Dev 2006; Izeradjene et al, Cancer Cell 2007
**P16/INK4a Conditional Knock-out Mice**

- **Why p16?**
  - Inactivated in virtually 100% of pancreatic cancer.
  - Germline mutations lead to increased risk for pancreatic cancer, melanoma, and head and neck cancer.

- **What is p16?**
  - An inhibitor of the cyclinD-Cdk4 and cyclinD-Cdk5 kinase complexes that down-regulate Rb.

- **Why conditional knock-out?**
  - P16 conventional knock-out mice die from lymphoma, sarcoma, melanoma etc. at early ages (Sharpless et al, Nature 2001).

**PKP GEMM-**

- **p16$^{lox/lox}$;LSL-**
- **Kras$^{G12D}$;Pdx1-Cre** mice

**p16 inactivation works synergistically with oncogenic Kras in promoting pancreatic tumorigenesis**

Medium survival of p16$^{-/-}$;LSL-Kras$^{G12D}$;Pdx1-Cre mice and p16$^{lox/lox}$;LSL-Kras$^{G12D}$;Pdx1-Cre (PKP) mice are 4 and 6 months respectively.

**PKP mice developed PDAC and frequent metastasis**

- PDAC is the major histologic presentation.
- Frequency of metastasis increased with age.
- Metastasis is observed in all non-thriving mice and involves liver, LN, stomach, lung, testis, spleen, etc.
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.

Mouse Model #2-IPMN/Pancreatic Ductal Adenocarcinoma

AKP GEMM - Acvr1bflox/flox; LSL-KrasG12D; Pdx1-Cre mice

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

Izeradjene et al, Cancer Cell 2007; Bardessy et. al., Can Dev 2006; Qiu & Su, Can Met Rev 2013

Mutational Profile of Pancreatic ductal adenocarcinoma (PDA)

ALK4/ACVR1B, a bona-fide tumor-suppressor gene

- Activin type 1 receptor B- part of the TGFbeta receptor family.
- Sequenced the genomic DNA of 29 (34% LOH) pancreatic cancer xenografts and 5 (45% LOH) pancreatic cancer cell lines genomic DNA in the ALK4 locus.
- Mutated in 2% of sporadic pancreatic ductal adenocarcinomas.

Su et al., PNAS: 98(6):3254-7, 2001

Inflammation and ADM were observed in the pancreata of Acvr1bf/f;Pdx1-Cre mice
AKP mice exhibited shortened survival and IPMN histologic phenotype

PanIN vs. IPMN

**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
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- Lesions greater than 1 cm in diameter
- Five-year survival following resection of IPMN with invasive cancer (43%) or without invasive cancer (77%)
- GNAS, RNF43, STK11, PIK3CA

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

Qiu et al., Gastroenterology, 2015

The Utilities of GEMMs
- Early Detection/Biomarkers- Prognosis, diagnosis, staging.
- Therapeutic targets
- Chemoprevention
- Tumorigenesis
- Cancer stem cells/tumor initiating niche
- Metastasis

Precision Medicine/Personalized Therapy

The Needs in PC Patient Care
- Early Detection-High-risk patients (familial and sporadic) and follow-up recurrence.
- Biomarkers-Prognosis, diagnosis, staging.
- Therapeutic targets
- Chemoprevention
- Animal modeling
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-Agbunag 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

Future Directions

- 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
- Cell Origin
  - PanIN vs. IPMN
  - Ductal vs. Acinar
- R01 Equivalent grants
  - Competing applications, awards, and success rates