Mouse Modeling for Human Pancreatic Cancer
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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 Nodeling 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 ovarian cancer, Dr. Mary-Claire King linked it to familial breast cancer.
- 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.

Cancer Statistics 2014
- A total of 1,665,540 new cancer cases and 585,720 deaths from cancer are projected to occur in the United States in 2014.
- Death rates peaked in 1991 (215.1 per 100,000 population) and up to 2010 have declined 20% (171.8 per 100,000).
- Over the past 10 years of available data (2000-2009), death rates continue to decline for all 4 major cancer sites (lung, colorectum, breast, and prostate); the largest annual declines in death rates were for chronic myeloid leukemia (8.4%), cancers of the stomach (3.1%) and colorectum (3.0%), and non-Hodgkin lymphoma (3.0%).
- The reduction in the overall cancer death rates translates to the avoidance of approximately 1.34 million deaths from cancer since 1991.

Epidemiology of Pancreatic Cancer
- Pancreatic cancer is relatively rare (the eleventh most common cancer in respect to incidence). >46,460 Americans are diagnosed with pancreatic cancer annually.
- The 5th leading cause of cancer death due to poor prognosis (~ 39,590 deaths estimated for 2014)- a five-year survival rate is ~6%.
- 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 genetic disease.
The pancreas is made up of three cell lineages-islet, acinar, and ductal epithelial cells. 

Pancreatic Cancer

- >75% of human pancreatic cancer are presented as ductal adenocarcinoma of the pancreas that progress from PanIN (pancreatic intraepithelial neoplasm) lesions.
- Others include: mucinous cystic neoplasms (MCNs), intraductal papillary mucinous neoplasms (IPMNs), medullary carcinoma, acinar cell carcinoma, pancreaticoblastoma etc.

Normal: The normal ductal and ductular epithelium is a cuboidal to low-columnar epithelium with amphophilic cytoplasm. Mucinous cytoplasm, nuclear crowding and atypia are not seen.
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 (7%).
  - Stage IV: The cancer has metastasized to the colon, stomach, or more distant organs such as the lungs or liver (7%).

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.
- 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%).

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).

Mutational Profile of Pancreatic ductal adenocarcinoma (PDA)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene Location</th>
<th>Frequency</th>
<th>Mutation Origin</th>
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<tbody>
<tr>
<td>BRCA1</td>
<td>13q12-13</td>
<td>30</td>
<td>som.</td>
</tr>
<tr>
<td>TP53</td>
<td>17p13</td>
<td>20</td>
<td>som.</td>
</tr>
<tr>
<td>KRAS</td>
<td>12q13</td>
<td>10</td>
<td>som.</td>
</tr>
</tbody>
</table>

Tumor Suppressor Genes Mutational Profile

- p16 9p21: 11q22
- p15 13q14
- p16 13q22
- CDH1 16q12
- SMAD4 18q21
- APC 5q21
- NF2 22q12
- TP53 17p13
- BRCA1 13q12
- BRCA2 13q12
- ATM 11q22
- TP53 17p13
- PTEN 10q23
- KRAS 12q13
- SMAD4 18q21

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Global genomic analysis of pancreatic cancer
(Jones et al, Science 2008)
- 24 pancreatic cancer, 23,219 transcripts, 20,661 genes, $\sim 10^6$
SNP-pancreatic cancer harbors 63 alterations on average, majority are point mutations.

The 63 alterations can be defined by 12 core signaling pathways.

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 ~251 million base pairs sequenced, corresponding to an average of 61 mutations per index metastatic lesion (range 41–77).
- Two categories of mutations were: ‘founder’ mutations present in all samples from a given patient; mean of 54%, 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.

Pancreatic cancer leave home early?
- Tagged pancreatic cells invaded and entered bloodstream early, before frank malignancy could be detected (Rhim et al, Cancer Cell 2012).
- Circulating tumor cells (CTC) were captured in 7/21 (33%) patients with cystic lesions and no clinical signs of cancer, 8/11 (73%) with PDA, and 0/19 (0%) of control (Rhim et al, Gastroentero 2014).

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
Hurdles in PC Research
- 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.
- Difficult to test chemopreventive therapeutics for high-risk patients.

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
- 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.

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
Orthotopic: Implantation into the pancreas

Carcinogen induced

Mouse Models for PC

Mouse Modeling for Human Pancreatic Cancer
**Genetically-Engineered Mouse Models**

- **Pro & Con**
  - Pro: Best mimicking human PC at genetic & histologic levels, intact TME, PanIN development, progression to PDA and metastasis.
  - Con: Expensive, time-consuming, labor-intensive, requires extensive knowledge on gene targeting.

- **Strategies**
  - Transgens
  - Knock-in
  - Knock-out

- **Targeted genes**
  - Oncogenes: mutant Kras, TGFalpha, SHH
  - TSG: p16, Smad4, p53, TGFbRII, Stk11, etc.

- **Targeted cell types**
  - Pancreatic progenitor cells
  - Acinar cells
  - Ductal epithelial cells
  - Centroacinar cells

**How to target pancreatic ductal epithelium?**

**Existing Mouse Models for Pancreatic Cancer**

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

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

**LSL-KrasG12D; Pdx1-Cre and LSL-KrasG12D; 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 KrasG12D 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)**
**LSL-Kras\(^{G12D}\) oncomice**

- Nestin-Cre;LSL-Kras\(^{G12D}\) mice - Same formation and frequency of PanIN as observed in Pdx1-Cre;LSL-Kras\(^{G12D}\)
- Pdx1-Cre;LSL-Kras\(^{G12D}\);p53\(^{R172H/+}\) mice have accelerated tumor progression, but the same histology-PanIN to PDA (Hingorani et al, Cancer Cell 2005).

**Pdx1-Cre; LSL-Kras\(^{G12D}\);Smad4\(^{-/-}\) mice preferentially develop mucinous cystic lesions in the pancreases (MCN vs IPMN)**

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


**Mouse Model #1**

PKP GEMM-

p16\(^{lox/lox}\);LSL-Kras\(^{G12D}\);Pdx1-Cre mice

**Mouse Model #2**

AKP GEMM-

Acrv1b\(^{lox/lox}\);LSL-Kras\(^{G12D}\);Pdx1-Cre mice

**Mouse Model #3**

STP GEMM-

Smad4\(^{lox/lox}\);MT-TGFalpha;p48-Cre mice