Genetics of Pancreatic Neuroendocrine Tumors

Saturday March 2, 2013

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The Sol Goldman Pancreatic Cancer Research Center
The Johns Hopkins Medical Institutions
Conflict of Interest

I receive royalty payments from Myriad Genetics for the PalB2 invention
Histologic Classification of Pancreatic Neoplasia

- Ductal Adenocarcinoma
- Neuroendocrine Tumor
- Acinar Carcinoma
- Intraductal Papillary Mucinous Neoplasm
- Mucinous Cystic Neoplasm
- Pancreatoblastoma
- Serous Cystadenoma
- Solid-pseudopapillary Neoplasm
Cancer is fundamentally a genetic disease.
Can we use this genetic revolution to classify pancreatic neoplasia, and if so where do pancreatic neuroendocrine neoplasms fit?
Can we translate genetic discoveries to patient care?
Adenocarcinoma

- 24 surgically resected ductal adenocarcinomas of the pancreas
- Sequenced over 750,000,000 base pairs of DNA from 20,661 genes
- Validated the findings in a separate set of 90 pancreatic cancers

Genetic Landscape of Pancreatic Cancer

Jones, et al., Science 2008
Genetic Landscape of Colon Cancer

Genetic Landscape of Pancreatic Cancer

CDKN2A
KRAS
TP53
SMAD4

Jones, et al., Science 2008
Can we translate genetic discoveries to patient care?
1. Prognosis
2. Risk Factors-
   Smoking
3. Familial Aggregation
4. Treatment
1. Prognosis- SMAD4

- Survival
  - Wild-type *SMAD4* – 14.2 months (95% CI -12.5-20.5)
  - Inactivated *SMAD4* – 11.5 months (95% CI – 8.5-16.0)
  - Cox Proportional Hazard Ratio – 1.92 (95% CI – 1.20 – 3.05; p = 0.006)

Blackford et al., Clin Cancer Research, 2009
15% Did Not Have Any Metastases

Iacobuzio-Donahue, J Clin Oncol. 2009 Apr 10;27(11):1806-13
Loss of SMAD4 expression in the primary carcinoma correlated with extensive metastatic burden (100-1000s) (P<0.002)

Iacobuzio-Donahue et al, J Clin Oncol. 2009 Apr 10;27(11):1806-13
2. Smoking

- Smoking causes one in four pancreatic cancers
- Examined the smoking-related genetic changes in all 21,000 human genes
- Ever Smokers had a mean of 75.5 mutations per tumor
- Never Smokers had a mean of 56.2 (p=0.04 for non-synonymous mutations)
- One in four mutations in the smokers was smoking related!!

A. Blackford, et al., Cancer Research 2009
3. Familial Aggregation

Family History of Pancreatic, Breast and Ovarian Cancer

Germline: 172-175 del TTGT

Somatic: IVS10 + 2 T>C
4. Treatment- Targeting Fanconi Anemia Mutations

Treated with Mitomycin C, Alive at 4 years!

Villarroel et al., Mol Cancer Ther. 2010
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Solid-pseudopapillary Neoplasms

- Arise in the tail of the pancreas in women
- Characterized by poorly cohesive cells
- The vast majority are cured by surgical resection, but ~10% metastasize

J. Wu et al, PNAS 2011
Solid-pseudopapillary Neoplasms

- **LOH**: Only one of the eight tumors studied exhibited any LOH.
- **Point mutations**: Only 2.9 point mutations per tumor (p<0.001 vs. the other cyst types).
- **Genes**: All 8 harbored mutations of *CTNNB1* (β-catenin).

Y. Tanaka, Cancer Res. 2001 Dec 1;61(23):840
Molecular Classification - Solid Pseudopapillary Neoplasms

Beta-catenin
Pancreatic Neuroendocrine Tumor with Hyaline Globules
Serous Cystadenoma

- Virtually all benign

J. Wu et al, PNAS 2011
Serous Cystadenoma

- **LOH**: Seven of the eight SCAs lost chromosome 3p alleles
- **Point mutations**: Average of 10 ± 4.6 nonsynonymous somatic mutations per tumor
- **Genes**: *VHL mutations* in 4 of 8, three of which had LOH at the *VHL* locus
Intraductal Papillary Mucinous Neoplasm

- Arise within the duct system
- Can progress to infiltrating adenocarcinoma

J. Wu et al, PNAS 2011
Intraductal Papillary Mucinous Neoplasm

- **LOH**: Four of the 8 IPMNs had LOH of chromosome 17q
- **Point mutations**: There were ~26 non-synonymous somatic mutations per tumor
- **Genes**: *RNF43* was mutated in six of the eight tumors, including all four that had 17q LOH
GNAS is an IPMN Gene

Two oncogenes were mutated in more than one IPMN

**KRAS (61%)** – G12D, G12R, G12V

**GNAS (81%)** – R201C, R201H

IPMNvs. Serous Cysts

Sequenced KRAS and GNAS in 176 Cysts
127 of 132 (96%) of IPMNshad a GNAS and/or a KRAS gene Mutation
All 44 Serous Cystadenomas were GNAS and KRAS Wild-type

Mucinous Cystic Neoplasm

- Much more common in women than in men
- Can progress to invasive carcinoma

J. Wu et al, PNAS 2011
Mucinous Cystic Neoplasm

- **LOH**: Only one region was lost in more than one tumor - chromosome 17q (the *RNF43* locus)
- **Point mutations**: There were ~16.0 non-synonymous somatic mutations per tumor, more than in SCAs and SPNs, but less than in IPMNs
- **Genes**: *RNF43*, *KRAS* and *TP53* (NOT *GNAS*)

![ Allele Ratio Graph with RNF43 highlighted ]
Cystic Neoplasms

J. Wu et al, PNAS 2011
Intraductal Papillary Mucinous Neoplasm

Serous Cystadenoma

A precursor to invasive pancreatic cancer

Virtually all are entirely benign
Real Clinical Problems

- Young mother
- 3.6 x 3.0-cm cyst in head of pancreas
  - Multiple loculated anechoic collections
  - No mural nodule or solid component
  - No ductal dilatation
- FNA performed
  No malignant cells

Courtesy of C. Wolfgang
The Cyst Got Bigger and Developed a Solid Component
Operation and Subsequent Course

- Pancreaticoduodenectomy
- Pathology
  - 3.2-cm serous cystadenoma
- Postoperative pancreatic fistula
- Severe diarrhea (6 months)
- “Eating difficulties” (3 months)
- Loss of energy and weight (3 months)
- Incisional hernia
Prevalence of Mutant GNAS in Pancreatic Juice by Diagnostic Group

Kanda, Gut 2012
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Pancreatic Neuroendocrine Tumors

1. More than 50% have liver metastases at diagnosis
2. 5-year survival rate 65%
   10-year 45%
3. An opportunity to compare the changes to those seen in ductal adenocarcinomas
“Next generation sequencers” can now be used to sequence entire human genomes in a matter of days.

Library Preparation: <6 h (<3 h hands-on)
Cluster Generation: <4 h (<10 min hands-on)
Sequencing by Synthesis: 1.5-8 days (<10 min hands-on)

Illumina.com
Two base-pair deletion in TP53
Pancreatic Neuroendocrine Tumors

1. Whole exomes of 10 PanNETs Sequenced using the Illumina GAIIX Platform (>18,000 genes)

2. Genes mutated two or more times sequenced using Sanger sequencing in a panel of 58 independent PanNETs

3. Immunolabeling for the protein products of key genes to demonstrate that the mutations are inactivating

Jiao, et al Science 2011
Three Mountains

1. **MEN-1** – 44% – Known before
2. **TSC2, PTEN** and **PIK3CA** – 16% - mTOR Pathway genes; A target for therapy
3. **DAXX** (death-domain associated protein) and **ATRX** (alpha thalassemia/mental retardation syndrome X-linked) – 45% - both are required for H3.3 incorporation in telomeres; A new cancer pathway

Jiao, et al Science 2011
PTEN Labeling of PEN104

Roeland de Wilde
## Neuroendocrine vs. Ductal

<table>
<thead>
<tr>
<th>Genes</th>
<th>PanNET</th>
<th>Adenocarcinoma</th>
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<tbody>
<tr>
<td>KRAS</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>TP53</td>
<td>3%</td>
<td>75%</td>
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<tr>
<td>CDKN2A</td>
<td>0%</td>
<td>95%</td>
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<td>SMAD4</td>
<td>0%</td>
<td>55%</td>
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<tr>
<td>MEN1</td>
<td>44%</td>
<td>0%</td>
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<tr>
<td>DAXX, ATRX</td>
<td>43%</td>
<td>0%</td>
</tr>
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<td>Genes in mTOR pathway</td>
<td>15%</td>
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Jiao, et al Science 2011
MEN-1 Labeling of PEN93

Roeland de Wilde
MEN-1

• **Gene**: Tumor suppressor gene on chromosome 11 (11q13)

• Ubiquitously expressed and highly conserved evolutionarily

• **Function**: diverse interactions suggest possible pivotal roles in transcriptional regulation, DNA processing and repair and cytoskeletal integrity

MEN-1

- Corbo et al looked at 169 PanNETs
  - 80% showed changes in expression
  - 30% harbored a mutation
  - mutations were distributed along the gene and most were of the loss of function type
- Germline mutations in families- MEN-1

Corbo et al, Endocr Relat Cancer, 2010
Multiple Endocrine Neoplasia-1 (Wermer Syndrome)

- Autosomal dominant inheritance
- Germline MEN-1 gene mutations
- Parathyroid adenoma (90%), pituitary adenoma, and entero-pancreatic endocrine tumors (40% gastrinoma, 10% insulinoma, 20% non-functional)

Gastrin     Glucagon       Insulin      PP      Somatostatin     Unclassified

Ln metas

Endocrine tumors

≥ 5 mm
< 5 mm - 1 mm
< 1 mm - 0.5 mm
< 0.5 mm

Klöppel et al, Cancer 1986
LOH Analysis in duodenal micro-NETs in MEN1:
Half of the neoplastic gastrin cells show LOH 11q13 (as small as 300 micron)

Anlauf et al Gut 2007

Endocrine cell hyperplasia in the duodenum

Endocrine tumors

- ≥ 5 mm
- < 5 mm - 1 mm
- < 1 mm - 0.5 mm
- < 0.5 mm
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Jiao, et al Science 2011
TSC2 Labeling of PEN93
Tuberous Sclerosis and PanNETs

- PanNETs have been reported in the tuberous sclerosis complex (TSC), but the incidence is low in comparison with other syndromes.
- Another nice example of “Knudson’s Hypothesis”

PanNET 93 with a TSC2 Gene Mutation

S. Albert et al, Expt. Opin. Invest. Drugs, 2010
PanNET 93 with a TSC2 Gene Mutation

S. Albert et al, Expt. Opin. Invest. Drugs, 2010
Targeting the mTOR Pathway

PanNET 31

PanNET 10

PanNET 93

S. Albert et al, Expt. Opin. Invest. Drugs, 2010

Everolimus

- Randomized trial of 410 patients (n=207 everolimus arm and n=203 for the placebo arm)
- 65% reduction in the estimated risk of progression or death

J. Yao, NEJM, 2011; 364:514-523
Modified from US News & World Report
Three Mountains

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Jiao, et al Science 2011
DAXX

Wild Type

Mutant
(A) Patients with a DAXX or ATRX gene mutation vs. patients in whom both genes were wild-type (WT) (Hazard Ratio 0.22, 95% CI 0.06 to 0.84, p = 0.03).

(B) Patients with mutations in MEN1 as well as either DAXX or ATRX vs. those in which all three genes were WT (Hazard Ratio 0.07, 95% CI 0.009 to 0.53, p=0.01).
Survival with Size

Data From the Johns Hopkins Database: Size of the tumor (p=0.0018)
Ki-67 Matters

Ki-67 < 2%
Ki-67 ~30%
Chromatin Regulators and Tumors

S J Elsässer et al. Science 2011;331:1145-1146

Published by AAAS
Normal Duct

• ALT+
  • ATRX mut, IHC Negative
  • DAXX wt, IHC Positive

• **ALT+**
  - ATRX wt, IHC Positive
  - DAXX mut, IHC Negative
DAXX/ATRX mutations Correlate with Alternative Lengthening of Telomeres.
A New Cancer Pathway: Solving the Chromosome End Replication Problem

http://www.henniker.org
One-third of neuroblastomas in adolescent and young adult group harbor \textit{ATRX} gene mutations.

Cheung, N. V. et al. JAMA 2012;307:1062-1071
Now that we have immunolabelling and FISH markers we can use them as probes to define the timing of genetic alterations.
Genetic Changes vs. Size

K. Matsukuma, Modern Pathology, 2012
ATRX/DAXX; ALT+ Occur Late

Microadenoma <0.5 cm

>3cm PanNET

K. Matsukuma, Modern Pathology, 2012
Neuroendocrine Carcinomas

• Defined by the presence of > 20 mitoses per 10 high power fields
• Divided into large cell endocrine carcinomas and small cell endocrine carcinomas
• Extremely aggressive
Ki-67 ~30%
Neuroendocrine Carcinoma

- 9 small cell NECs, 10 large cell NECs, and 11 well-differentiated neuroendocrine tumors (PanNETs) of the pancreas
- Abnormal immunolabeling patterns of p53 and Rb were frequent (p53, 18 of 19, 95%; Rb, 14 of 19, 74%) in both small cell and large cell NECs, whereas DAXX, and ATRX labeling was intact in virtually all of these same carcinomas.

Summary of Small Cell Carcinoma

- *TP53* and *Rb* are targeted in both small cell and large cell neuroendocrine carcinomas
- *DAXX*, and *ATRX* are intact
- Small cell carcinomas are not simply advanced well-differentiated neuroendocrine tumors
Careful integration of genetics, IHC and morphology can lead to a better understanding of a disease and can have significant clinical implications.
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## Classification of Pancreatic Neoplasia

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<th>KRAS</th>
<th>TP53</th>
<th>RB</th>
<th>SMAD4</th>
<th>p16</th>
<th>MEN1</th>
<th>DAXX</th>
<th>ATRX</th>
<th>mTOR</th>
<th>β-cat</th>
<th>GNAS</th>
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Tumor abbreviations:
- PanNET: Pancreatic Neuroendocrine Tumor
- Small Cell
- SPN: Solid Pseudopapillary Neoplasm
- Adeno: Acinar Cell Neuroendocrine Tumor
- IPMN: Intraductal Papillary Mucinous Neoplasm
- MCN: Mucinous Cystic Neoplasm
- Serous
A better understanding of the fundamental mechanisms driving pancreatic neuroendocrine tumorogenesis will be translated to improve patient care
Mourning Dove Track and Field
The Hurdles
Thank You!