NEXT GENERATION LEARNING

2016 ANNUAL MEETING
March 12-18 Seattle, Washington
New Developments in the Genetics of Pancreatic Cancer: Nature’s Heart Beats Strong Amid the Hills

March 13, 2016

Ralph H. Hruban, M.D.
Professor of Pathology and Oncology
The Sol Goldman Pancreatic Cancer Research Center
The Johns Hopkins Medical Institutions
DISCLOSURES

I receive royalty payments from Myriad Genetics for the *PalB2* invention.
Major Sequencing Studies

2. Biankin et al - 100 exomes in 2012
3. Waddell et al - 100 genomes in 2015
5. Roberts et al - 638 germline genomes - 2016

Jones et al. Science. 2008;321: 1801-6
Biankin; Nature. 2012; 491: 399–405
Waddell; Nature 2015
Witkiewicz, Nat Commun. 2015; 6:6744
Roberts, Cancer Discovery, 2016
24 surgically resected ductal adenocarcinomas of the pancreas

Sanger sequenced over 750,000,000 base pairs of DNA from 20,661 genes

Validated the findings in a separate set of 90 pancreatic cancers

Total of 114 ductal adenocarcinomas

Genetic Landscape of Ductal Adenocarcinoma: Jones et al

Jones, et al., Science 2008
Tragedy of the Lar de Gaube

“Nature's heart beats strong amid the hills.”

- Richard Monckton Milnes (Lord Houghton)

Bull Run

Some things stand out on hills
DNA Mismatch Repair System (MMR)

3p: MLH1

Jones, et al., Science 2008
Microsatellite Instability

Medullary Carcinoma

1. Poorly differentiated
2. Syncytial growth pattern
3. Pushing boarders
Loss of MLH1
Microsatellite Instability (MSI)

1. MS status has prognostic value- median survival for MSI-high cases of 62 months, versus 10 months (hazard ratio = 5.6; P = 0.007)

2. MS status has implications for other family members. The medullary phenotype is highly associated with a family history of cancer (P<0.001).

3. MSI-high cancers respond to immunotherapy!

Nakata et al., Clin Cancer Res; 8: 2536-40.
Microsatellite Instability and Response to Pembrolizumab

Dung T. Le et al., NEJM 2015
Microsatellite Instability and Response to Pembrolizumab

Dung T. Le et al., 2015
Microsatellite Instability

The presence of multiple mutations = Recognizable by the immune system -> Immunotherapy
Some families are defined by hills

Marktschellenberg, Germany

Sound of Music Hill
(Marktschellenberg, Germany)

Familial Genomes

- Sequenced 638 germline genomes from patients with familial pancreatic cancer
- Confirmed previously identified familial pancreatic cancer susceptibility genes such as BRCA2, CDKN2A and ATM, and identified novel candidate familial pancreatic cancer genes, such as BUB1B.

Roberts et al, Cancer Discovery 2016
PacGene Validation

- 727 unrelated probands with positive family history (521 met criteria for familial pancreatic cancer)
- \( BRCA2 \), 3.7%; \( CDKN2A \), 2.5%; \( BRCA1 \), 1.2%; \( PALB2 \), 0.6%.
- More germline mutations in the four genes (8.0%) in patients with a family history of pancreatic cancer than in non-familial pancreatic cancer probands (3.5%).
- \( BRCA2 \) and \( CDKN2A \) account for the majority of mutations in familial pancreatic cancer.
Genetic Landscape of Familial Pancreatic Cancer

Roberts et al, Cancer Discovery, 2016
BRCA2 and Other Fanconi Anemia Genes

Jones, et al., Science 2008
BRCA2 - Familial Risk of Pancreatic Cancer

• Carriers of the 6174 del T BRCA2 mutation have ~10x increased risk of developing pancreatic cancer

• ~3-7% of patients with “sporadic” pancreatic cancer have germline BRCA2 mutations

• ~12% of patients with 2 relatives with pancreatic cancer have a germline BRCA2 mutations

• ~17% of patients with 3 relatives with pancreatic cancer have a germline BRCA2 mutations

Cancer Res 56:5360
Cancer Res 62:3789-3793
Nat Genet 16:17
BRCA2- Genomic Instability

• Whole-genome sequencing and copy number variation (CNV) analysis of 100 ductal adenocarcinomas

• Variations in chromosomal structure were used to classify the tumors into 4 subtypes: stable, locally rearranged, scattered and unstable.

• Genomic instability co-segregated with inactivation of DNA maintenance genes (BRCA1, BRCA2 or PALB2) and with a mutational signature of DNA damage repair deficiency.

Waddell; Nature 2015
**BRCA2- Genomic Instability**

(a) Stable, Scattered, Unstable, Locally rearranged

- Intra-chromosomal rearrangement
- Inter-chromosomal translocation
- Duplication
- Tandem duplication
- Inversion
- Foldback inversion
- Amplified inversion
- Deletion

(b) BRCA mutations per Mb

- Upper quintile
- BRCA mutational signature

- Category positive
- Somatic mutation (non-silent)
- Somatic mutation (silent)
- Structural variant

- LOH

Participants:
- BRCA1
- BRCA2
- PALB2
- ATM
- RPA1
- REV3L
- TP53

Waddell; Nature 2015
BRCA2 and Therapy

D'Andrea A D Genes Dev. 2003;17:1933-1936

Cold Spring Harbor Laboratory Press
Metastatic Pancreatic Cancer in a Patient With a Germline BRCA1 (5385insC) Mutation Treated with Cisplatin and a PARP Inhibitor

Before

After

Courtesy Eileen O’Reilly
Exceptional responder
ICGC_0006
Unstable / somatic BRCA2 biallelic
BRCA signature rank 14
Complete radiological and CA19.9 response

CA 19.9 (U per ml)

Surgery Recur
Adjuvant gemcitabine
Platinum therapy

Upper limit of normal
Alive

Time (months)

Waddell; Nature 2015
<table>
<thead>
<tr>
<th>Individual</th>
<th>% of Families</th>
<th>Increased Risk</th>
<th>Age 50</th>
<th>Age 70</th>
</tr>
</thead>
<tbody>
<tr>
<td>No History</td>
<td>-</td>
<td>1</td>
<td>0.05%</td>
<td>0.5%</td>
</tr>
<tr>
<td>Hereditary Non-polyposis Colorectal Cancer</td>
<td>?</td>
<td>8</td>
<td>1%</td>
<td>3.7%</td>
</tr>
<tr>
<td>BRCA2 (Breast-Ovarian)</td>
<td>6-12%</td>
<td>3.5-10</td>
<td>0.5-2%</td>
<td>5%</td>
</tr>
<tr>
<td>PALB2</td>
<td>3%</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Familial Atypical Multiple Mole Melanoma (p16)</td>
<td>1-3%</td>
<td>20-34</td>
<td>1%</td>
<td>10-17%</td>
</tr>
<tr>
<td>Familial Pancreatitis (PRSS1)</td>
<td>&lt;1%</td>
<td>50-80</td>
<td>2.5%</td>
<td>25-40%</td>
</tr>
<tr>
<td>Peutz-Jeghers (STK11/LKB1)</td>
<td>&lt;1%</td>
<td>132</td>
<td>6.6%</td>
<td>30-60%</td>
</tr>
<tr>
<td>ATM</td>
<td>&lt;2%</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>
Some hills define real danger!

Karakoram Highway, Pakistan

http://www.wonderslist.com/10-most-dangerous-roads-in-the-world/
Sequenced the Exomes of Cystic Precursor Neoplasms of the Pancreas
Cystic Neoplasms

J. Wu et al, PNAS 2011
Validation-Scarpa

- 48 intraductal papillary neoplasms
- Targeted panel of 51 genes
- GNAS and/or KRAS mutations were found in 44/48 (92%) of IPMNs
- RNF43 was the third most commonly mutated gene
- TP53 and SMAD4 in high-grade dysplasia

Scarpa et al, J Pathol 2014; 233: 217–227
Prevalence of Mutant GNAS in Pancreatic Juice Collected at Endoscopy Matches that in IPMNs

Kanda, Gut 2012
A Tool for Pathologists: Incipient IPMNs

- 21 lesions in the grey zone between PanIN and IPMN (<1cm)
- Mutational analysis revealed KRAS codon 12 mutations in all 21 lesions, and 7 (33%) harbored GNAS codon 201 mutations.
- The presence of GNAS mutations in these lesions suggests that a fraction of these are in fact small IPMNs (incipient IPMNs).

A Tool for Pathologists: IPMNs -> Ca

A Tool for Pathologists: Multifocal IPMN

---

Little Round Top, Gettysburg

Some Hills Can Be Targeted

http://www.totalgettysburg.com/little-round-top.html
**Targeted Therapy**

- *RNF43* is targeted in IPMNs
- RNF43 plays a role in the Beta-catenin pathway.
- RNF43 removes surface Wnt (wingless) receptors.

Proc Natl Acad Sci U S A. 2015;112:7548-50
Targeted Therapy

- Targeting the β-catenin pathway (Porcupine inhibitor [C59]) may be a way to treat IPMN-associated (RNF43 dependent) invasive cancers.

Proc Natl Acad Sci U S A. 2015;112:7548-50
Targeted Therapy

- **ARID1A, MLL, MML2, MLL3 mutated** in 20% of pancreatic cancers.
- Gordon Mills et al have reported that **ARID1A mutations** confer sensitivity to PARP inhibitors in cell lines.

Jones et al, Hum Mutat 33:100–103, 2012
Nat Commun 2015, 6:7686
Targeting the mTOR Pathway in Pancreatic Neuroendocrine Tumors

PanNET 31

PanNET 10

PanNET 93

= Mutation site

Baylor Group

- Sequenced 109 micro-dissected cancers
- *KRAS* mutations are observed in >90% of cases, and were mutually exclusive with oncogenic *BRAF* mutations
- *BRAF* mutations defined sensitivity to vemurafenib in pancreatic cancer models

Nat Commun. 2015 Apr 9;6:6744
GALAPAGOS

Darwin’s Finches

Charles Darwin

• "The distribution of tenants of this archipelago would not be nearly so wonderful, if for instance, one island has a mocking-thrush and a second island some other quite distinct species... But it is the circumstance that several of the islands possess their own species of tortoise, mocking-thrush, finches, and numerous plants, these species having the same general habits, occupying analogous situations, and obviously filling the same place in the natural economy of this archipelago, that strikes me with wonder."

Journal written on the Beagle 1831-1835
Thank You!

@Hopkins_GI_Path