Neoplastic progression in macroscopic precursor lesions of the pancreas

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Elizabeth Thompson reported no relevant financial relationships

USCAP staff associated with the development of content for this activity reported no relevant financial relationships.
Outline

- Brief overview of pancreatic ductal adenocarcinoma (PDAC) genetics
- Classification of macroscopic precursor lesions in the pancreas
  - Histology
  - Molecular features
    - Impact on understanding of PDAC biology and therapy
- Harnessing molecular features of precursor lesions for pre-op diagnosis
  - Cyst fluid analysis
Genetic landscape of pancreatic cancer

Jones, et al., Science 2008

CDKN2A, KRAS, TP53, SMAD4
The PDAC Genome Now

• Several groups have reported whole exome or whole genome sequencing of PDACs – >600 exomes analyzed in total!


• KRAS, CDKN2A, TP53, SMAD4 remain the “mountains”

• Numerous “hills” also identified

• Subgroups defined based on mutation signatures and structural rearrangements
Familial pancreatic cancer genes – very heterogeneous
- BRCA2, PALB2, CDKN2A, STK11, ATM, and possibly BRCA1
- up to 4% of apparently sporadic PDAC have deleterious germline mutations, most commonly in BRCA2 (Shindo et al, JCO 2017)
- Germline testing part of NCCN guidelines for PDAC

Homology-directed repair (HDR) defects
- Leads to genomic instability
- Can lead to sensitivity to platinum agents and PARP inhibitors (POLO trial)

MSI-H
- Very small hill in PDAC ~ 1%
- Important for prognosis, prediction of familial risk and therapy

KRAS wild-type
- May be driven by other oncogene alterations, some of which may be targetable – BRAF mutations, ERBB2 amp, NRG fusions

Immune checkpoint blockade
Precursor lesions in the pancreas

- Series of genetic and accompanying histologic changes that precede the development of an invasive neoplasm with the potential to metastasize

  - Pancreatic intraepithelial neoplasia (PanIN)
  - Intraductal papillary mucinous neoplasm (IPMN)
  - Mucinous cystic neoplasm (MCN)

    Share many genetic alterations

  - Intraductal oncocytic papillary neoplasm (IOPN)
  - Intraductal tubulopapillary neoplasm (ITPN)
Intraductal papillary mucinous neoplasm (IPMN)

- Grossly visible ($\geq 1$ cm), mucin producing epithelial neoplasm
- Grows within main pancreatic duct or side branches
- Frequent papillary architecture
- Slight male predominance
- Head $>$ Tail
- Growth along native pancreatic ducts
Involvement of main and side branch ducts
IPMN with low-grade dysplasia

- Polarized, minimal variation in nuclear size and shape, no complex architecture
IPMN with high-grade dysplasia

- Loss of polarization, nuclear size variation, complex architecture
Mucinous cystic neoplasm (MCN)

- Mucin-producing cyst with ovarian-type stroma
- Much more common in women than in men (10:1)
- Mean age at diagnosis: ~50
- Tail > Head
- Typically don’t communicate with pancreatic duct system
Mucinous cystic neoplasm (MCN)
Mucinous cystic neoplasm (MCN)

| Estrogen Receptor | Progesterone Receptor |
MCN with low-grade dysplasia

- Cyst-budding/daughter cysts very common in MCN
MCN with high-grade dysplasia
Intraductal papillary mucinous neoplasm genetics

- 81% have hotspot mutations in **KRAS** (codon 12)
- 61% have hotspot mutations in **GNAS** (codon 201)
  - 96% had GNAS and/or KRAS mutation
- 75% have inactivating mutations in **RNF43**, 50% have LOH at RNF43 locus on chr 17q
- **CDKN2A**
- **TP53**
  
  - Other identified mutations:
    - **KLF4** – low-grade dysplasia
    - **PI3K** and **WNT** signaling pathway
    - **ATM**
    - **GLI3** (Hedgehog pathway)
    - **MUC16**
    - **STK11**
    - **PTPRT**
    - **CNTN5**
    - **ERBB2** and **MYC** amplification

Germline mutations in IPMN patients

- Study of 350 patients with resected IPMN
  - 7.3% had germline mutation associated with cancer risk
  - 2.9% had germline mutations with known PDAC association
    - ATM, PTCH1, SUFU

- Patients with IPMN + germline mutations associated with PDAC were more likely to have concurrent invasive carcinoma

- Utility of screening patients with known IPMN for germline mutations—potentially identify a group at high risk for progression?

Skaro et al, Gastroenterology 2019
Mucinous cystic neoplasm genetics

- Many shared mutations with IPMN:
  - KRAS
  - CDKN2A
  - RNF43
  - PIK3CA
  - TP53
- No GNAS alterations

Garcia-Carracedo et al, Pancreas 2014; Noe et al, Nat Commun 2020
**Intraductal papillary mucinous neoplasm genetics**

- Single-cell sequencing from multiple regions of a unifocal IPMN

- Different mutations in the same early driver gene (KRAS, GNAS) occur in different cells within the same IPMN

- Multiple mutations in later-occurring driver genes (RNF43, CDKN2A) were also common and localized to unique tumor clones

- Reveals remarkable genetic heterogeneity with single IPMNs
- Suggests a much more complex pattern of tumor evolution

Intraductal papillary mucinous neoplasm genetics

Retrospective targeted-sequencing of multiple regions of low- and high-grade dysplasia encompassing each representative FFPE block of 20 IPMN

- Low-grade regions characterized by multiple mutations in early driver genes (KRAS, GNAS) representing independent, spatially separate clones

- High-grade regions lack heterogeneity in early driver genes but have multiple mutations in later driver genes

- Suggestive of polyclonal origin of IPMN – but could multiple KRAS mutations have been later divergent events in a monoclonal IPMN?
- Select whole exome sequencing confirmed lack of shared mutations in different regions with mutually exclusive early driver genes

Emerging picture of polyclonal evolution in IPMN

• Suggests convergent evolution in transition from low- to high-grade dysplasia
• Challenges traditional view of monoclonal origin of pancreatic neoplasms

• What drives the selection for (and possibly against) certain clones allowing for their propagation?
• Risk stratification of patients?

Figure from editorial on previous work: Jacob and Banerjee, Gastroenterology 2019.
Malignant progression from precursor to invasive carcinoma

- 148 samples from IPMNs, MCNs, and small associated invasive carcinomas (18 patients)
- Analyzed with whole exome or targeted sequencing
- Compared mutations and used evolutionary analyses to link genetic alterations in the non-invasive presumed precursor lesions and adjacent invasive carcinomas

Noe et al, Nat Commun 2020
Evolutionary reconstruction of the phylogeny establishes IPMN and MCN as precursors of associated invasive carcinomas. Estimates using Bayesian hierarchical models for number of acquired mutations over a range of mutation rates. Time interval between high-grade dysplasia development and PDAC founder cell ranges from ~3-7 years with average median time of 3.7 years.

Noe et al, Nat Commun 2020
Additional (surprising? confounding?) data

- IPMN and associated invasive carcinomas are not always genetically related “neighbors but not relatives”
  - Felsenstein et al, Gut 2018; Omori et al, Gastroenterology 2019

- Lesions smaller than 1 cm (gross definition of IPMN) can harbor GNAS mutations → “incipient” IPMN
  - Matthaei et al, AJSP 2014

- When analyzed in 3D across completely sectioned FFPE blocks, IPMN can be surprisingly large
  - Ongoing work at JHH: Ashley Kieman, Pei-Hsun Wu, Alicia Braxton, Laura Wood, Ralph Hruban, Denis Wirtz
PanIN in 3D

Slide courtesy of Ashley Kiemen, with additional credit to Pei-Hsun Wu, Alicia Braxton, Laura Wood, Ralph Hruban, Denis Wirtz
Intraductal oncocytic papillary neoplasm (IOPN)

- Formerly classified as an oncocytic subtype of IPMN
- Voluminous granular eosinophilic cytoplasm; nuclei with single prominent nucleolus
- Complex arborizing papillae
- Mucin is not prominent, may have intracytoplasmic vacuoles
- Essentially all show high-grade dysplasia
Intraductal oncocytic papillary neoplasm (IOPN)

- Complex, arborizing papillae with abundant eosinophilic cytoplasm
Intraductal oncocytic papillary neoplasm (IOPN)

- Complex, arborizing papillae with abundant eosinophilic cytoplasm and single prominent nucleolus, lack of prominent mucin
Intraductal oncocytic papillary neoplasm (IOPN)

- About 60% of IOPN will label for HepPar-1
Intraductal oncocytic papillary neoplasm (IOPN)

- Formerly called an oncocytic variant/subtype of IPMN

- But – different genetics
  - No KRAS or GNAS mutations
  - ARHGAP26, ASXL1, EPHA8, and ERBB4 alterations have been reported

- Recently, recurrent rearrangements in PRKACA and PRKACB identified in 100% of a cohort of 20 IOPN
  - ATP1B1-PRKACB, DNAJB1-PRKACA, and ATP1B1-PRKACA fusions identified

IPMN can have oncocytic areas – but neoplasms with oncocytic + mucinous areas with gastric/intestinal differentiation shown to have KRAS, GNAS alterations

Same fusion seen in fibrolamellar hepatocellular carcinoma

Basturk et al, Mod Pathol 2016; Singhi et al, Gastroenterology 2020
Intraductal tubulopapillary neoplasm (ITPN)

- Predominant growth as large intraductal nodules of complex back-to-back tubules and anastomosing papillary formations
  
  - Cuboidal cells with minimal cytoplasm
  - No obvious intracellular mucin
  - Central necrosis is common
  - Pattern of growth can resemble DCIS and/or papillary carcinoma of breast

- Can be difficult to determine exact size and extent of associated invasive carcinomas → about 60-70% will harbor invasion with 5 year survival 70%

Intraductal tubulopapillary neoplasm (ITPN)

- Complex back-to-back glands and anastomosing papillae
Intraductal tubulopapillary neoplasm (ITPN)

- Complex back-to-back glands and anastomosing papillae; central necrosis
Intraductal tubulopapillary neoplasm (ITPN)

- Complex back-to-back glands and anastomosing papillae; minimal cytoplasm, no obvious mucin
Intraductal tubulopapillary neoplasm (ITPN)

- Genetics distinct from IPMN –
  - no KRAS, BRAF, only rare SMAD4 loss, p16, P53 alterations
  - Somatic mutations have been seen in chromatin remodeling genes and PI3K pathways members
  - FGFR2 fusions also identified

Insights from genetics of different cystic precursor lesions

- How does our broadening understanding of neoplastic progression in precursor cysts impact our perspectives on PDAC behavior, therapy and diagnosis?
  - IPMNs very genetically heterogeneous – implications for attempts at preoperative diagnosis and patient prognostication

- Window of likely several years between initiation of high-grade dysplasia and invasive carcinoma → opportunity both for patient identification and potentially therapeutic intervention
  - Cancers arising out of IPMN, IOPN, ITPN likely genetically different, behave differently, different therapy? Targets for IOPN?

- Cancers adjacent to IPMN may be genetically related or unrelated

- Major opportunity for pre-operative diagnosis – cyst fluid analysis

Poster Plug: Progression of immune response in matched IPMN and invasive carcinomas
Preoperative cyst diagnosis – unmet clinical need

- 70 million CT scans performed in the US each year

- In one study, ~2.5% asymptomatic patients undergoing abdominal CT had pancreatic cyst
  - Laffan et al, AJR 2008

- Possible diagnoses include benign cysts and precursors to invasive cancer – operate or observe?
  - Creates a major challenge for clinical management
  - Criteria exist to guide observation vs. resection: Sendai, etc
  - Recommendations by major GI clinical associations may give differing advice
Whole exome sequencing of pancreatic cysts revealed a unique mutation profile for each type.
Mutational analysis of cyst fluid can identify cyst type!
A panel of molecular markers + clinical features classifies cyst type with 90-100% sensitivity and 92-96% specificity

- Springer et al, Gastroenterology 2015

Preoperative NGS of cyst fluid for KRAS/GNAS mutations is highly sensitive for IPMNs and specific for mucinous cysts

Combination of TP53/PIK3CA/PTEN alterations is a useful preoperative marker for advanced neoplasia.

- Singhi et al, Gut 2017

Algorithm combining clinical features, imaging characteristics, and cyst fluid genetics and biochemical markers “CompCyst” was more accurate in guiding clinical management than standard of care using pathology at resection as gold standard diagnosis

- Springer et al, Sci Transl Med 2019
Challenges in cyst fluid analysis

- Spatial and genetic heterogeneity preset real challenges to accurate patient stratification
- Genetics more complicated than one mutation per gene
- Potential more complex additional metrics: number of mutations, clonality, VAF, etc
- Continued addition of additional alterations as they are identified
## Genetics of precursor lesions in the pancreas

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Description</th>
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<tbody>
<tr>
<td>IPMN</td>
<td>Molecular studies continue to add to our understanding of precursor lesions but also reveals ever increasing complexity polyclonality, challenging traditional monoclonal models of pancreatic neoplasia development</td>
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<tr>
<td>MCN</td>
<td>Window between high-grade dysplasia and development of invasive carcinoma leaves room for clinical detection and intervention</td>
</tr>
<tr>
<td>IOPN</td>
<td>Unique molecular alterations between different cyst types can be harnessed for preoperative diagnosis using cyst fluid</td>
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THANK YOU!