Molecular Detection of Advanced Neoplasia in the Pancreas

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Objectives

• Discuss knowledge gaps in pancreatic cancer screening

• Present the results of two sequential studies aimed
  • To discover and validate novel methylated DNA biomarkers for detecting pancreatic high-grade dysplasia and cancer in tissue
  • To assess and compare distributions of methylated DNA markers in pancreatic cyst fluid
Pancreatic Cancer

- 4th → 3rd leading cause of cancer death in U.S.
  - 2nd by 2020

- Life-time risk
  - Average risk: 1.5%
  - Hereditary syndromes: 5-50%

- 5-year survival
  - All stages combined: 8%
  - Stage 1 (sx): 27%
  - Stage 1 (pre-sx, <1 cm): 75%

- No screening
Screening Targets

Pre-cancer

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

Cancer

• Early stage
Pancreatic Cystic Neoplasms

• Incidentally detected pancreatic cysts are common
  • Prevalence on CT/MRI 2.4%-19.6%
• Majority do not harbor advanced dysplasia
  • Do not justify surgery
• No reliable method to detect advanced neoplasia
• Uncertainty about diagnosis and malignant potential leads to
  • Frequent surveillance and high health care cost
  • Unnecessary surgery
Progression of Dysplasia

Cystic neoplasms: 2-tier classification system*
- Low-grade dysplasia (LGD)
- High-grade dysplasia (HGD)

PanIN
- PanIN 1A, 1B, and 2: LGD
- PanIN 3: HGD

Progression of Dysplasia: When to intervene?

Cysts with advanced dysplasia
- Greatest risk of progression to invasive cancer
- Resection at stage of HGD
  - Excellent long term survival similar to cysts harboring LGD
- No reliable approach to discriminate HGD vs LGD prior to surgery

Current Approaches

Cystic neoplasms
• Cyst morphology
  • Sendai guidelines
    • Low specificity ~20%
  • Fukuoka guidelines
    • Specificity 73%
    • Sensitivity 56%
• Cyst fluid analysis
  • CEA
    • Does not detect dysplasia/cancer
  • Cytology
    • Low sensitivity
    • Poor discrimination for dysplasia

PanINs
• Cannot be visualized

Future approaches
• Molecular markers?
  • Genetic
  • Epigenetic

DNA Methylation

• Single predictable target site
  • Promoter region
• Highly informative
  • Single or combination of a small number of markers can achieve AUC close to 1
• Readily available assay platforms
• FDA-approved multi-target stool DNA test for colon cancer
Tissue study
Aims

To identify and validate methylated DNA marker candidates that distinguish

Case group
- IPMN-HGD
- PanIN-3
- Cancer

Control group
- Normal pancreas
- IPMN-LGD
- PanIN-1 & 2
Discovery

- Unbiased whole methylome sequencing
  - Reduced representation bisulfite sequencing (RRBS)
    - Cancer vs normal
      - Frozen
    - LGD vs HGD
      - Paraffin-embedded

- 25 candidate markers selected
  - AUCs $\geq 0.85$, fold change $\geq 25$, $P \leq 0.01$

- 3 pancreatic epithelial markers
  - Present in cases and controls, not in leukocytes
Validation

Technical
• Methylation-specific PCR (MSP)
• 23 of 25 markers retained

Biological
• Independent tissue set
• Blinded MSP assay
Biological Validation

Case group (n=53)
- IPMN-HGD (23)
- PanIN-3
- Cancer (30)

Control group (n=111)
- IPMN-LGD (36)
- PanIN-1 (44)
- PanIN-2
- Normal (31)
## Top 10 Markers

<table>
<thead>
<tr>
<th>Marker</th>
<th>AUC</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBX15</td>
<td>0.89</td>
<td>0.83-0.95</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VWC2</td>
<td>0.89</td>
<td>0.83-0.95</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PRKCB</td>
<td>0.89</td>
<td>0.83-0.95</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CLEC11A</td>
<td>0.89</td>
<td>0.83-0.94</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EMX1</td>
<td>0.88</td>
<td>0.82-0.94</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ELMO1</td>
<td>0.88</td>
<td>0.81-0.94</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DLX4</td>
<td>0.87</td>
<td>0.81-0.92</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ABCB1</td>
<td>0.87</td>
<td>0.80-0.93</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ST8SIA1</td>
<td>0.87</td>
<td>0.80-0.93</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SP9</td>
<td>0.86</td>
<td>0.79-0.92</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Marker Distributions
Cases vs Controls

TBX15

VWC2

PRKCB

Standardized copy number

Cases
Controls

Normal LGD HGD IPMN 1 2 3 Cancer

Normal LGD HGD IPMN 1 2 3 Cancer
Discrimination by 4-Marker Panel

Specificity  Sensitivity
98%       73%
89%       84%

AUC=0.90 (0.84, 0.96)
Cyst fluid study
Aims

1. To assess and compare distributions of methylated DNA markers in cyst fluid that distinguish:
   - HGD
   - Cancer

2. To compare methylated DNA marker distributions in cyst fluid with those of CEA and mutant KRAS:
   - LGD
   - No dysplasia
Methods

• 0.2 ml cyst fluid from surgically resected cysts
• DNA extracted and bisulfite converted
• Methylated DNA marker (MDM) panel selected from
  • Previous technical and biological validation in tissue
  • Pilot study for optimization of marker assay in cyst fluid
• Methylation-specific PCR (MSP)
Methods

- **KRAS mutations**
  - Quantitative allele-specific PCR

- CEA analysis
  - Commercial kit (MILLIPLEX® MAP Kit)

- Fukuoka risk stratification was performed using pre-operative imaging
  - Negative
  - Worrisome
  - High risk
Study Groups

Cases (n=21)
- HGD (8)
  - IPMN
  - MCN
- Cancer (13)

Controls (n=113)
- LGD (68)
  - IPMN
  - MCN
- No dysplasia (45)
  - SCA
  - Pseudocyst
  - Others
## Demographic characteristics

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong>#</td>
<td>71 (56,77)</td>
<td>61 (46,69)</td>
</tr>
<tr>
<td><strong>Male</strong>#</td>
<td>11 (61%)</td>
<td>31 (32%)</td>
</tr>
<tr>
<td><strong>Smoking</strong></td>
<td>10 (48%)</td>
<td>43 (38%)</td>
</tr>
<tr>
<td><strong>Personal history of non-pancreatic cancer</strong></td>
<td>5 (28%)</td>
<td>14 (15%)</td>
</tr>
<tr>
<td><strong>Family history of pancreatic cancer</strong></td>
<td>1 (6%)</td>
<td>7 (7%)</td>
</tr>
<tr>
<td><strong>Collection Method (Surgery)</strong></td>
<td>18 (86%)</td>
<td>81 (83%)</td>
</tr>
</tbody>
</table>

# P<0.05
Dysplasia distribution across Fukuoka risk categories

Majority of resected cysts do not harbor advanced neoplasia
Marker distributions: Top 3 markers

- **TBX15**
- **BMP3**
- **CD1D**
Performance of 3-marker cyst fluid MDM panel

TBX15, BMP3, and CD1D

Specificity 90%
Sensitivity 86%

AUC 0.93
MDM panel compared to KRAS and CEA

<table>
<thead>
<tr>
<th>Marker</th>
<th>Sensitivity % @ 90% Specificity</th>
<th>AUC (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDM Panel</td>
<td>86</td>
<td>0.93 (--o)</td>
</tr>
<tr>
<td>KRAS</td>
<td>29</td>
<td>0.71 (0.0007)</td>
</tr>
<tr>
<td>CEA</td>
<td>48</td>
<td>0.72 (0.0035)</td>
</tr>
</tbody>
</table>

- **MDM Panel**: 86% sensitivity with 0.93 AUC (P-value: --)
- **KRAS**: 29% sensitivity with 0.71 AUC (P-value: 0.0007)
- **CEA**: 48% sensitivity with 0.72 AUC (P-value: 0.0035)
Marker distribution across Fukuoka risk strata

<table>
<thead>
<tr>
<th>Strata</th>
<th>Controls</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>80%</td>
<td>80%</td>
</tr>
<tr>
<td>Worrisome</td>
<td>60%</td>
<td>60%</td>
</tr>
<tr>
<td>High Risk</td>
<td>40%</td>
<td>12%</td>
</tr>
</tbody>
</table>

TBX15

<table>
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Conclusions

In pancreatic cyst fluid, novel methylated DNA markers:

• Accurately discriminate cases (HGD/cancer) from controls (LGD/normal)

• Exhibit significantly superior sensitivity and specificity for advanced neoplasia compared to current clinical risk prediction models and biomarkers

Further optimization and investigations are indicated to corroborate and extend findings
Acknowledgement
Thank you