Precursor lesions in humans and mouse models

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Pancreatic pathology: Of mice and men
Madrid, December 4-6th 2014
Introduction

Precursor - Definition

• Criteria to define a precursor to invasive cancer:
  • Must be associated with an increased risk of the cancer
  • Resulting cancer arises from cells within the precursor
  • Precursors differ from the normal tissue
  • Precursors differ from the cancer
  • A method should be available by which the precursor can be diagnosed
• Comparable morphology in humans and mouse models
Introduction

Precursors of PDAC

- Pancreatic Intraepithelial Neoplasia (PanIN)
- Intraductal papillary mucinous neoplasia (IPMN)
- Mucinous cystic neoplasia (MCN)
- (Atypical Flat Lesions (AFL))
Pancreatic Intraepithelial Neoplasia (PanIN)

- Microscopic (<0.5cm)
- Flat or papillary
- Noninvasive
- Arise in small intra- or interlobular pancreatic ducts
- Number increases with age
- Male=Female
- Head>Body/Tail
- Often multifocal

- Ductal epithelial proliferation
- confined to the native pancreatic ducts (<1mm)
- appropriate setting
- no significant acinar differentiation

Occur in several mouse models (chemically induced and GEMM)
Pancreatic Intraepithelial Neoplasia (PanIN)

Noninvasive epithelial proliferations within the smaller pancreatic ducts

**PanIN1A**: flat with uniform, tall columnar cells, basally located nuclei and abundant supranuclear mucin, nuclei small, basally located, round to oval

**PanIN1B**: morphologically identical to PanIN1A, but with papillary, micropapillary or basally pseudostratified architecture

Common genetic alterations:
- Telomere shortening
- Activating point mutation of KRAS
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**Humans vs. mouse models**
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Humans vs. mouse models

PAS-reaction

PAS-Alcianblue staining
Pancreatic Intraepithelial Neoplasia (PanIN)

Noninvasive epithelial proliferations within the smaller pancreatic ducts

**PanIN2**: flat or papillary with some nuclear abnormalities (loss of polarity, nuclear crowding, enlarged nuclei, pseudostratification or hyperchromatism), rarely mitoses

**Common genetic alterations:**
- Telomere shortening
- Activating point mutation of KRAS
- Inactivating mutations in p16/CDKN2

Maitra et al. Mod Pathol 2003
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**PanIN3:** papillary or micropapillary, cribriform with loss of polarity, dystrophic goblet cells, mitoses (normal/abnormal), nuclear irregularities, prominent (macro) nucleoli; “budding off” into the lumen, luminal necrosis; no invasion through the basement membrane

**Common genetic alterations:**
- Telomere shortening
- Activating point mutation of *KRAS*
- Inactivating mutations in *p16/CDKN2*
- Inactivation of *SMAD4, TP53* and *BRCA2*
Pancreatic Intraepithelial Neoplasia (PanIN)

Noninvasive epithelial proliferations within the smaller pancreatic ducts

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**Humans vs. mouse models**
Human PanIN

Low-grade

High-grade

Ki67

p53
Low-grade mPanIN

mPanIN 1A

CK19: red
Amylase: brown

Ki-67

mPanIN 1B
High-grade mPanIN

CK19: red
Amylase: brown

Ki-67
Pancreatic Intraepithelial Neoplasia (PanIN)

Noninvasive epithelial proliferations within the smaller pancreatic ducts

Humans vs. mouse models

- Is distinguished from IPMN by size (<1cm) and morphology
- “Cancerization” of ducts can mimic PanIN

- Appropriate setting (confined to native pancreatic ducts, <1mm)
Pancreatic Intraepithelial Neoplasia (PanIN)

Noninvasive epithelial proliferations within the smaller pancreatic ducts

Humans vs. mouse models

- Is distinguished from IPMN by size (<1cm) and morphology
- „Cancerization“ of ducts can mimic PanIN

- Appropriate setting (confined to native pancreatic ducts, < 1mm)
- Ductular-insular complexes should not be classified as PanINs
IPMN

Humans vs. mouse models

- Grossly visible mucin-producing epithelial neoplasm in main pancreatic duct or one of its branches
- Papillary architecture

- Cystic papillary neoplasms
  - Cystic structures >1mm
  - Papillary, noninvasive epithelial proliferations with varying degrees of cellular atypia
  - Might resemble human IPMN

Described in models with:
- Concomitant expression of TGFα and Kras$^{G12D}$
- Additional deletion of Smad4 in Cre-Kras$^{G12D}$ model
IPMN

Humans vs. mouse models

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Intestinal type

Pancreatobiliary type

Kras$^{G12D}$-Ela TGFα
Ptf1a^{+}/Cre;Kras^{G12D};Ela-Tgfa

Siveke et al, Cancer Cell 2007
MCN

Humans vs. mouse models

- Mucin-producing epithelial cells associated with an ovarian-type of stroma
- Different degrees of epithelial dysplasia
- Stroma expresses progesterone and estrogen receptors and inhibin
- Associated carcinoma in about 30% of MCN

- Cystic neoplasms with mucinous epithelium
- With dense “ovarian-type” stroma
- Presence/absence of associated invasive carcinoma should be documented
- Occurred after additional Smad4 or Notch2 deletion in KC model
Precursors of PDAC in mouse models

A

Activation

Inactivation

Cre recombinase (defines cell lineage)

STOP

B

Kras

+ Tgα3
+ Smad4
+ Notch2

IPMN

MCN

PanIN

PanIN

PanIN

PanIN

IPMN-to-PDAC

MCN-to-PDAC

Poorly-differentiated PanIN-to-PDAC

Anaplastic PanIN-to-PDAC

Metastatic PanIN-to-PDAC

Well-differentiated PanIN-to-PDAC

Mazur, Siveke; Gut 2011
Acinar ductal metaplasia (ADM)

- Metaplasia = Conversion or replacement of one differentiated cell type with another
- ADM: acinar cells undergo metaplasia to a ductal cell phenotype in a setting of acute or chronic inflammation
- Occurrence of tubular complexes (TC) and mucinous tubular complexes (MTC)

Humans vs. mouse models

- ADM progressively extends with increasing age (in Ptf1Cre;KrasG12D – mice about 77% of whole pancreas replaced with 36 weeks)
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ADM

Kras
Notch
TGFα
Rac1
Raf/MEK/ERK
...

inflammation

Tubular complexes

De La O et al, PNAS 2008
Siveke et al, Cancer Cell 2007
Guerra et al, Cancer Cell 2007
Heid et al, Gastroenterology 2011
Shi et al, Oncogene, 2013
Ptf1a\textsuperscript{+/Cre(ex1), LSL-Kras\textsuperscript{G12D}} (4 weeks)

Amylase: brown
CK19: red
ADM in the human pancreas
ADM in the human pancreas

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Nº</th>
<th>TC or</th>
<th>PanIN</th>
<th>TC</th>
<th>PanIN-1</th>
<th>PanIN-2</th>
<th>PanIN-3</th>
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<tr>
<td>PDAC</td>
<td>92</td>
<td>72 (78%)</td>
<td>56 (61%)</td>
<td>54 (59%)</td>
<td>17 (18%)</td>
<td>14 (15%)</td>
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<tr>
<td>CP</td>
<td>45</td>
<td>42 (93%)</td>
<td>35 (78%)</td>
<td>32 (71%)</td>
<td>5 (11%)</td>
<td>0</td>
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</tr>
<tr>
<td>SCA</td>
<td>27</td>
<td>18 (67%)</td>
<td>12 (44%)</td>
<td>16 (59%)</td>
<td>4 (15%)</td>
<td>0</td>
<td></td>
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</tbody>
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<table>
<thead>
<tr>
<th>Fall</th>
<th>ADM</th>
<th>ADM</th>
<th>PanIN-1</th>
<th>PanIN-2</th>
<th>PanIN-3</th>
<th>Karzinom</th>
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<tbody>
<tr>
<td>1</td>
<td>CGT</td>
<td>CGT</td>
<td>GAT</td>
<td>Wildtyp</td>
<td>GAT</td>
<td>GAT</td>
</tr>
<tr>
<td>2</td>
<td>GAT</td>
<td>GAT</td>
<td>GAT</td>
<td>–</td>
<td>–</td>
<td>GAT</td>
</tr>
<tr>
<td>3</td>
<td>CGT + GTT</td>
<td>Wildtyp</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>GTT</td>
</tr>
<tr>
<td>4</td>
<td>GAT + GTT</td>
<td>GAT</td>
<td>CGT</td>
<td>–</td>
<td>–</td>
<td>GAT</td>
</tr>
<tr>
<td>5</td>
<td>GTT</td>
<td>GTT</td>
<td>GAT + GTT</td>
<td>GAT</td>
<td>GAT</td>
<td>GAT</td>
</tr>
<tr>
<td>6</td>
<td>CGT + GTT</td>
<td>CGT</td>
<td>GAT</td>
<td>GTT</td>
<td>GAT</td>
<td>GAT</td>
</tr>
</tbody>
</table>

*Kras*

Atypical flat lesions (AFL)

Ptft1a+/Cre(ex1);LSL-KrasG12D/+
Atypical Flat Lesions (AFL)

- Flat, non-mucinous lesions
- Background of ADM with dysplasia (nuclear pleomorphy, increased nuclear-cytoplasm ratio, prominent nucleoli, mitoses)
- perilesional stromal reaction
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PAS-reaction

PAS-Alcianblue staining
Atypical Flat Lesions (AFL)

Table 4. Molecular analysis of microdissected murine lesions

<table>
<thead>
<tr>
<th>Sample</th>
<th>p16^{nk4} methylation</th>
<th>p16^{nk4a} gene inactivation</th>
<th>p19^{Arf} gene inactivation</th>
<th>p53 gene mutation</th>
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</thead>
<tbody>
<tr>
<td>M222</td>
<td>+</td>
<td>N</td>
<td>N</td>
<td>N</td>
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<tr>
<td>W36</td>
<td>+</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>M172</td>
<td>+/-</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>W270</td>
<td>+/-</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
</tbody>
</table>

Y = yes; N = no. +/−, weak promoter methylation detected; +, robust promoter methylation detected.
Kinetics of the lesions

Number of lesions/mouse vs. Age (weeks)

- Black squares: AFL
- Gray square: PanIN 3
<table>
<thead>
<tr>
<th>Marker</th>
<th>TC MML</th>
<th>MTC'/ AFL</th>
<th>PanIN low-grade</th>
<th>PanIN high-grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylase</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Muc1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Claudin-18</td>
<td>+</td>
<td>+</td>
<td>-/+</td>
<td>+</td>
</tr>
<tr>
<td>Mib1</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>p53 (TP53)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Smad 4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>p16*</td>
<td>nd</td>
<td>nd</td>
<td>-</td>
<td>nd</td>
</tr>
<tr>
<td>Pdx1</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
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<tr>
<td>CK5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>αSMA</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
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Aichler et al, *J Pathol* 2012
Esposito et al, *Pathologe* 2012