Molecular genetics of PDAC

Irene Esposito, Melissa Schlitter

Pancreatic pathology: Of mice and men
Madrid, December 4-6th 2014
**Background:** Molecular genetic analysis of DNA from paraffin-embedded PDAC tissue

DNA isolation from FFPE tissue
Milestones in pancreatic cancer biology

Genetic fingerprint of PDAC
- KRAS
- SMAD4
- p16
- TP53

12 core signalling pathways

Molecular subtypes of PDAC
- classical
- exocrine
- QM

Different prognosis
Distinct drug response

Personalized treatment of PDAC
- resection/biopsy
- molecular analysis
- personalized therapy

1997
2008
2011
Future

Esposito et al., World J Gastroenterol 2014
The genetic fingerprint of PDAC

42 cases

- 2 mutations (15%)
- 1 mutation (8%)
- 4 mutations (38%)
- 3 mutations (38%)

The genetic fingerprint of PDAC/founder mutations

Rozenblum et al., Cancer Res. 1997
The genetic fingerprint of PDAC

<table>
<thead>
<tr>
<th></th>
<th>Iacobuzio-Donahue 2012</th>
<th>Schlitter, Segler, Esposito</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRAS</td>
<td>95 %</td>
<td>91 %</td>
</tr>
<tr>
<td>P16</td>
<td>90 %</td>
<td>78 %</td>
</tr>
<tr>
<td>TP53</td>
<td>75 %</td>
<td>70 %</td>
</tr>
<tr>
<td>SMAD4</td>
<td>55 %</td>
<td>38 %</td>
</tr>
</tbody>
</table>

Iacobuzio-Donahue et al., Clin Canc Res 2012
Schlitter et al unpublished data
Milestones in pancreatic cancer biology

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1997 2008 2011 Future
Core Signaling Pathways in Human Pancreatic Cancers Revealed by Global Genomic Analyses

Siân Jones1,*, Xiaosong Zhang1,*, D. Williams Parsons1,2,*, Jimmy Cheng-Ho Lin1,*, Rebecca J. Leary1,*, Philipp Angenendt1,*, Parminder Mankoo3, Hannah Carter3, Hirohiko Kamiyama4, Antonio Jimeno1, Seung-Mo Hong4, Baojin Fu4, Ming-Tseh Lin4, Eric S. Calhoun1, Mihoko Kamiyama4, Kimberly Walter4, Tatiana Nikolskaya5, Yuri Nikolsky6, James Hartigan7, Douglas R. Smith7, Manuel Hidalgo1, Steven D. Leach1,8, Alison P. Klein1,4, Elizabeth M. Jaffee1,4, Michael Goggins1,4, Anirban Maitra1,4, Christine Iacobuzio-Donahue1,4, James R. Eshleman1,4, Scott E. Kern1,4, Ralph H. Hruban1,4, Rachel Karchin3, Nickolas Papadopoulos1, Giovanni Parmigiani1,9, Bert Vogelstein1,†, Victor E. Velculescu1,†, and Kenneth W. Kinzler1,†
Global genomic analysis of PDAC

- **Deletion**
  loss of genetic material; part of a chromosome or a sequence of DNA is missing

- **Amplification**
  mechanism leading to multiple copies of a chromosomal region within a chromosome arm

- **Mutation**
  change in the sequence of an organism's genetic material.
Central role of KRAS in PDAC

• early event: present in PanIN lesions (>90% low-grade)
• PDAC: mutated KRAS in 70-100%
• most common mutation G12V → permanent activation

• $p48^{+/Cre}$; $Kras^{+/LSL-G12D}$ mouse model (Hingorani et al., 2003)
  → pancreas specific inactivation of Kras → pancreatic cancer
Loss of p16/CDKN2A in PDAC

- Deletion

![Image of gel electrophoresis with bands for Exon 1 and Exon 2 showing a comparison between normal (N) and tumor (T) samples for p16 and Globin.](image)

- Promoter hypermethylation
- Point mutation
- Germline mutations in FAMMM (familiar atypical mole malignant melanoma)

Esposito et al. 2004
**TP53 mutations**

- Mostly missense mutations of DNA-binding domain
- Later event (progression low grade to high grade)
- Correlation with survival
- Important for sensitivity/resistance to radio-/chemotherapy

Pellegata et al., Cancer Res. 1994  
Nakamori et al., Jpn. J. Cancer Res. 1995  
Luttges et al., Am. J Pathol. 2001  
Giovanetti et al., Mol. Cancer Ther. 2006
SMAD4 (tumor suppressor gene)

- Deleted in Pancreatic Cancer 4 (DPC4)
- Located on chromosome 18q21
- Deletion or point mutation
- Prevalence 30-50%
- Late event in carcinogenesis
- Correlation with survival and metastasis

Iacubuzio-Donahue, J Clin Oncol 2009
Oshima et al., Ann Surg 2013
Milestones in pancreatic cancer biology

- **1997**: Genetic fingerprint of PDAC
  - KRAS
  - SMAD4
  - p16
  - TP53

- **2008**: 12 core signalling pathways

- **2011**: Molecular subtypes of PDAC
  - Classical
  - Exocrine
  - QM
  - Different prognosis
  - Distinct drug response

- **Future**: Personalized treatment of PDAC
  - Resection/biopsy
  - Molecular analysis
  - Personalized therapy
LETTERS

Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy

Eric A Collisson^1,2,10, Anguraj Sadanandam^1,3,10, Peter Olson^4,9, William J Gibb^1,9, Morgan Truitt^4, Shenda Gu^1, Janine Cooc^5, Jennifer Weinkle^1, Grace E Kim^6, Lakshmi Jakkula^1, Heidi S Feiler^1, Andrew H Ko^2, Adam B Olshen^7, Kathleen L Danenberg^5, Margaret A Tempo^2, Paul T Spellman^1, Douglas Hanahan^3,4 & Joe W Gray^1,8
Milestones in pancreatic cancer biology

1997
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Future
Wild type KRAS is associated with significantly better prognosis

Overall survival analysis (n=164*)

<table>
<thead>
<tr>
<th></th>
<th>KRAS wt</th>
<th>KRAS mut</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-year survival*</td>
<td>92.9%</td>
<td>64.8%</td>
<td></td>
</tr>
<tr>
<td>2-year-survival*</td>
<td>71.4%</td>
<td>38.0%</td>
<td></td>
</tr>
<tr>
<td>3-year-survival*</td>
<td>59.5%</td>
<td>26.3%</td>
<td>p=0.01</td>
</tr>
<tr>
<td>Median survival</td>
<td>n.e.</td>
<td>18.8 months</td>
<td></td>
</tr>
</tbody>
</table>

Log rank test p=0.009

Wild type KRAS is associated with significantly better survival estimations

*Excluded for overall survival analysis:
M1-status (n=10), lost to FU (n=1), and peroperative death (n=1)
**PDAC: from bench to bedside**

**Basic research**
- Identification of genomic alteration and changes in protein expression

**Clinical research**
- Integration of clinical data (survival, follow-up)

- **genetic marker for clinical outcome**
  e.g. *TP53, SMAD4*

- **therapeutic targets**
  e.g. EGFR

- **predictor of response to therapy**
  e.g. molecular subtypes Collission et al.
„Microenvironment“

<20% cancer cells

1. Defense reaction of the host?
2. Primary genetic stromal defect?
3. Stromal activation by mutated epithelial cells?

>80% stroma
1. Frühe Aktivierung der Stromareaktion

α-SMA

Tenascin C

Kollagen V

β6-Integrin

Annexin 2

β1-Integrin

Esposito et al, J Pathol 2006
Berchtold et al, in Vorbereitung
Steiger et al, in Vorbereitung
2. Epithel-Stroma Interaktionen in der frühen Karzinogenese

TNC beeinflusst die β6-integrin Expression

Ptf1a<sup>+/Cre(ex1)</sup>;LSL-Kras<sup>G12D</sup>

Ptf1a<sup>+/Cre(ex1)</sup>;LSL-Kras<sup>G12D;TNC<sup>−/−</sup>-mice</sup>

TNC beeinflusst das epitheliale „Remodelling“

ADM/PanIN/AFL

% of ductal structures

Steiger et al, in Vorbereitung
Haneder et al, in Vorbereitung

3 Monate alt
3. Epithel-Stroma Interaktionen in der Tumorprogression

Rot: Kollagen V Grün: α-SMA

Berchtold et al, in Vorbereitung
Höhere Progressionsrate der Patienten, die mit Saridegib + Gemcitabin behandelt wurden im Vergleich zu den Kontrollen

Kürzeres medianes Überleben im Vergleich zum historischen medianen Überleben von Patienten, die mit Gemcitabin allein für 6 Monate behandelt wurden
### Antifibrotic therapy of pancreatic cancer

<table>
<thead>
<tr>
<th>Rank</th>
<th>Status</th>
<th>Study</th>
<th>Condition</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Not yet recruiting</td>
<td>A Biomarker Study to Identify Predictive Signatures of Response to LDE225 (Hedgehog Inhibitor) In Patients With Resectable Pancreatic Cancer</td>
<td>Pancreatic Ductal Adenocarcinoma</td>
<td>Drug: LDE225</td>
</tr>
<tr>
<td>2</td>
<td>Recruiting</td>
<td>Hedgehog Inhibitors for Metastatic Adenocarcinoma of the Pancreas</td>
<td>Metastatic Pancreatic Cancer</td>
<td>Drug: Gemcitabine, nab-Paclitaxel, GDC-0449</td>
</tr>
<tr>
<td>4</td>
<td>Not yet recruiting</td>
<td>Effect on Tumor Perfusion of a Chemotherapy Combining Gemcitabine and Vismodegib Before Surgery in Pancreatic Cancer</td>
<td>Pancreatic Adenocarcinoma Resectable</td>
<td>Drug: gemcitabine; Drug: Vismodegib; Procedure: Neoadjuvant chemotherapy</td>
</tr>
<tr>
<td>5</td>
<td>Completed</td>
<td>A Study Evaluating IPI-926 in Combination With Gemcitabine in Patients With Metastatic Pancreatic Cancer</td>
<td>Metastatic Pancreatic Cancer</td>
<td>Drug: IPI-926 plus gemcitabine; Drug: Placebo plus gemcitabine</td>
</tr>
<tr>
<td>6</td>
<td>Active, not recruiting</td>
<td>GDC-0449 and Erlotinb Hydrochloride With or Without Gemcitabine Hydrochloride in Treating Patients With Metastatic Pancreatic Cancer or Solid Tumors That Cannot Be Removed by Surgery</td>
<td>Adenocarcinoma of the Pancreas, Recurrent Pancreatic Cancer, Stage IV Pancreatic Cancer, Unspecified Adult Solid Tumor, Protocol Specific</td>
<td>Drug: vismodegib; Drug: erlotinb hydrochloride; Drug: gemcitabine hydrochloride; Other: diagnostic laboratory biomarker analysis</td>
</tr>
<tr>
<td>7</td>
<td>Terminated</td>
<td>Hedgehog Inhibition for Pancreatic Ductal Adenocarcinoma (PDAC) in the Preoperative Setting (HIPPoS)</td>
<td>Pancreatic Ductal Adenocarcinoma</td>
<td>Drug: GDC-0449</td>
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<td>8</td>
<td>Active, not recruiting</td>
<td>Vismodegib and Gemcitabine Hydrochloride in Treating Patients With Advanced Pancreatic Cancer</td>
<td>Recurrent Pancreatic Cancer, Stage IV Pancreatic Cancer</td>
<td>Drug: vismodegib; Drug: gemcitabine hydrochloride; Other: laboratory biomarker analysis</td>
</tr>
<tr>
<td>9</td>
<td>Active, not recruiting</td>
<td>Gemcitabine Hydrochloride With or Without Vismodegib In Treating Patients With Recurrent or Metastatic Pancreatic Cancer</td>
<td>Adenocarcinoma of the Pancreas, Recurrent Pancreatic Cancer, Stage IV Pancreatic Cancer</td>
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Stromal Elements Act to Restrain, Rather Than Support, Pancreatic Ductal Adenocarcinoma

1. Wirtsreaktion (Barriere)?
2. Primärer genetischer Stromadefekt?
3. Aktivierung des Stromas durch bereits mutierte epitheliale Zellen?