How does pancreatic cancer develop?
Implications for treatment

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Pancreatic cancer

- early diagnosis of ductal adenocarcinoma is rare
- at diagnosis 10 - 20 % of cancers are resectable, < 1 % curable
- wide resection is impossible
- perineural infiltration, early metastasis to lymph nodes, peritoneum and liver
- ductal phenotype, intense desmoplastic reaction
- no sensitivity to chemotherapy or radiotherapy
How does pancreatic cancer develop?

- Environmental / genetic causes?
- Genetic alterations in pancreatic cancer specimens?
- Precancerous lesions?
- Molecular mechanisms of initiation of carcinogenesis?
- Cell of origin of pancreatic cancer?
- Genetic pathways to pancreatic cancer development?
- Epigenetic changes?
- Mechanisms of metastasis?
How does pancreatic cancer develop?

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  - Mechanisms of metastasis?
How does pancreatic cancer develop?

Textbook

- genetic 10%
- smoking 30%
- nutrition 20%
- benign diseases 4%
- unknown 36%
How does pancreatic cancer develop?

- Start of disease
- Neoplasia
- Invasion
- Metastasis

Birth → Start of disease → Neoplasia → Invasion → Metastasis → Death
How does pancreatic cancer develop?

environment

- start of disease
- neoplasia
- invasion
- metastasis

birth

death
How does pancreatic cancer develop?

- Smoking / obesity
- Start of disease
- Neoplasia
- Invasion
- Metastasis
- Birth
- Death
How does pancreatic cancer develop?

- environment
  - start of disease
    - neoplasia
    - invasion
    - metastasis
  - death
- birth

- genetic predisposition
Genetic predisposition - monogenic diseases

- FAMMM
- Peutz-Jeghers syndrome
- FAP
- HNPCC
- BRCA2-associated carcinoma
- Ataxia teleangiectasia
- Li-Fraumeni syndrome
- Hereditary pancreatitis

CDKN2 (p16)

STK11 (LKB1)

APC

MSH2, MLH1

BRCA2

ATM

TP53 (p53)

PRSS1
Genetic predisposition – family history

- FAMMM  \( CDKN2 \) (p16)
- Peutz-Jeghers syndrome  \( STK11 \) (LKB1)
- FAP  \( APC \)
- HNPCC  \( MSH2, MLH1 \)
- BRCA2-associated carcinoma  \( BRCA2 \)
- Ataxia teleangiectasia  \( ATM \)
- Li-Fraumeni syndrome  \( TP53 \) (p53)
- Hereditary pancreatitis  \( PRSS1 \)
- Familial pancreatic cancer
Familial pancreatic cancer - Carter Family
Familial pancreatic cancer

• **5-fold** increased risk with 2 first degree relatives with pancreatic cancer

• **18-fold** increased risk with > 2 first degree relatives with pancreatic cancer

• **57-fold** increased risk with > 3 first degree relatives with pancreatic cancer

Tersmette et al 2001, Ghardirian et al 2002
How does pancreatic cancer develop?

- Environment
  - Start of disease
    - Neoplasia
    - Invasion
    - Metastasis
  - Birth
  - Death
- Monogenic/polygenic
Risik groups - pancreatic cancer

How does pancreatic cancer develop?

- **smoking**
  - start of disease
  - neoplasia
  - invasion
  - metastasis

- **birth**
- **death**

- **family history**
Risik groups - pancreatic cancer

How does pancreatic cancer develop?

- Environmental / genetic causes?
- Genetic alterations in pancreatic cancer specimens?
- Precancerous lesions?
- Molecular mechanisms of initiation of carcinogenesis?
- Cell of origin of pancreatic cancer?
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- Mechanisms of metastasis?
How does pancreatic cancer develop?

- *birth*

- *start of disease*

- *neoplasia*

- *invasion*

- **diagnosis:** resectable pancreatic cancer

  - Whippel
Structural changes in pancreatic cancer specimens

- **KRAS** mutations 100 %
- **INK4a** (p16) inactivation 82 %
- **Tp53** (p53) inactivated 76 %
- **SMAD4** (DPC4) inactivated 53 %

Rozenblum E et al 1997
Tumo-suppressive pathways in Pancreatic cancer

- KRAS mutations additional tumor suppressor
  - p16, p53, and DPC4 inactivation: 15 (3x)
  - p16 and p53 inactivated: 10
  - p16 and DPC4 inactivated: 5 (2x)
  - p53 and DPC4 inactivated: 0
  - only p53 inactivated: 4 (1x)
  - only p16 inactivated: 2
  - only DPC4 inactivated: 0
  - wild-type p16, p53, and DPC4: 2

Rozenblum E et al 1997
How does pancreatic cancer develop?

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How does pancreatic cancer develop?

- start of disease
- neoplasia
- invasion

birth

?
Genetic alteration in PanINs

PanIN-1A       PanIN-1B       PanIN-2       PanIN-3

Pancreatic Intraepithelial Neoplasia
## Genetic alteration in PanINs

<table>
<thead>
<tr>
<th>PanIN-1A</th>
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<tbody>
<tr>
<td>Telomeric length</td>
<td>KRAS</td>
<td>CDKN2/INK4A (p16)</td>
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PanIN-3 is genetically advanced

SMAD4 (DPC4)
Genetic alteration in PanINs

PanIN-1A       PanIN-1B        PanIN-2         PanIN-3
expression 47 438 578
changes (screened 21329)

Buchholz et al 2005

pancreatic cancer: 610 expression changes
PanIN-2 is the earliest truly preneoplastic lesions

Buchholz et al 2005
How does pancreatic cancer develop?

50% at the age of 50 harbor PanIN-1B lesions
How does pancreatic cancer develop?

- Environmental / genetic causes?
- Genetic alterations in pancreatic cancer specimens?
- Precancerous lesions?
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How does pancreatic cancer develop?

start of disease → neoplasia → invasion

birth → Kras?
Pancreatic development

organ-
specification

stem
cell

Progenitor
cell

differentiated
cell

PDX-1

Ptf1

(Ptf1a = p48)

endocrine

ductal

exocrine
Pancreatic cancer development

organ-specification  stem cell  progenitor cell  differentiated cell

PDX-1  
Ptf1

(Ptf1a = p48)

ductal pancreatic cancer cell

endocrine

ductal

exocrine
Expression of $Kras^{G12V}$ in CK19 positive cells
Expression of $\text{Kras}^{G12V}$ in CK19 positive cells

A: wild type
B: CK19-$\text{Kras}^{G12V}$
C: anti-CD4
D: ductal hyperplasia

Brembeck et al 2003
Expression of $Kras^{G12V}$ in CK19 positive cells

differentiated cell
endocrine
ductal
exocrine

Expression of $Kras^{G12V}$ in CK19 positive cells

no PanINs
no tumors
infiltration with CD4$^+$ lymphocytes
Expression of \( \text{Kras}^{G12D} \) in elastase positive cells

- differentated cell
  - endocrine
  - ductal
  - exocrine

\[ \text{CK19} \rightarrow \text{Kras}^{G12V} \rightarrow \text{no PanINs, no tumors, infiltration with CD4^+ lymphocytes} \]

\[ \text{elastase} \downarrow \text{Kras}^{G12D} \rightarrow ? \]
Expression of $Kras^{G12D}$ in elastase positive cells

- **D**: hyperplastic acini
- **E**: tubular complexes, increased stroma
- **K**: 16 months old, intraductal papillary mucinous carcinoma (IPMT)
- **M**: CK19

Grippo et al 2003
Expression of \( \text{Kras}^{G12D} \) in elastase positive cells

differentiated cell

- endocrine
- ductal
- exocrine

\[ \text{CK19} \quad \text{Kras}^{G12V} \quad \text{no PanINs} \quad \text{no tumors} \quad \text{infiltration with CD4+ lymphocytes} \]

\[ \text{elastase} \quad \text{Kras}^{G12D} \quad \text{no PanINs} \quad \text{tubular complexes} \quad \text{acinar-to-ductal metaplasia} \quad \text{IPMT} \]
Activation of $Kras^{G12D}$ in pancreatic stem cells

organ-specification  stem cell

PDX-1  Ptf1  $Kras^{G12D}$  ?

(Ptf1α = p48)
Activation of $\text{Kras}^{G12D}$ in pancreatic stem cells

$\text{WT}$

$\text{Kras}^{G12D}$

$\text{PDX-1-Cre or Ptf1-Cre}$

$\text{WT}$

$\text{Kras}^{G12D}$
Activation of $\text{Kras}^{\text{G12D}}$ in pancreatic stem cells

Hingorani et al. 2003
Activation of $Kras^{G12D}$ in pancreatic stem cells

A: wild type; B: reactive duct; C and D: PanIN-1A; E and F: PanIN-1B

Hingorani et al 2003
Activation of $Kras^{G12D}$ in pancreatic stem cells

$G$ and $H$: PanIN-2; $I$ to $L$: PanIN-3

Hingorani et al 2003
Activation of $Kras^{G12D}$ in pancreatic stem cells

Hingorani et al. 2003
Activation of $Kras^{G12D}$ in pancreatic stem cells

I to K: liver

L to N: lung

O: lymph node
P: diaphragma
Q: perineural

Hingorani et al 2003
Activation of $Kras^{G12D}$ in pancreatic stem cells

organ-specification stem cell

PDX-1 Ptf1

$Kras^{G12D}$

(Ptf1α = p48)

PanIN-1-3 ductal pancreatic carcinoma
typical metastasis perineural infiltration
How does pancreatic cancer develop?

- Environmental / genetic causes?
- Genetic alterations in pancreatic cancer specimens?
- Precancerous lesions?
- Molecular mechanisms of initiation of carcinogenesis?
- **Cell of origin of pancreatic cancer**?
- Genetic pathways to pancreatic cancer development?
- Epigenetic changes?
- Mechanisms of metastasis?
Origin of pancreatic cancer

Organ specification → Stem cell

PDX-1, Ptf1

Kras\textsuperscript{G12D}

progenitor cell → ductal pancreatic cancer cell

PDX-1

Ptf1

(Ptf1a = p48)
...mutations convert normal stem-cell self-renewal pathways into engines for neoplastic proliferation...

...specific gene products regulate both the self-renewal of normal somatic stem cells and the proliferation of cancer stem cells...
How does pancreatic cancer develop?

- Environmental / genetic causes?
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- **telomeric length**
- **KRAS**
- **CDKN2/INK4A** (p16)
- **TP53** (p53)
- **SMAD4 (DPC4)**
Tumo-suppressive pathways in Pancreatic cancer

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<th>38 tumor suppressors</th>
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<tr>
<td>p16, p53, and DPC4 inactivation</td>
<td>15</td>
</tr>
<tr>
<td>p16 and p53 inactivated</td>
<td>10</td>
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Rozenblum E et al 1997
How does pancreatic cancer develop?

Start of disease → Neoplasia → Invasion

Birth → Kras → Ink4a?
Activation of \( Kras^{G12D} \) and inactivation of \( Ink4a/Arf \) in pancreatic stem cells

organ-specification -> stem cell

PDX-1
Ptf1

\( Kras^{G12D} \)
\( Ink4a/Arf^{-/-} \)

(Ptf1a = p48)
Activation of $\textit{Kras}^{G12D}$ and inactivation of $\textit{Ink4a}/\textit{Arf}$ in pancreatic stem cells

Aguirre et al 2003

sarcomatoid well differentiated

well differentiated poorly differentiated

sarcomatoid

CK19 CK19 CK19

Aguirre et al 2003 CK19 CK19 CK19
Activation of $\text{Kras}^{G12D}$ and inactivation of $\text{Ink4a/Arf}$ in pancreatic stem cells

A: duodenal invasion
B: lymph node metastasis
C: invasion in stomach wall
D: micrometastasis in the portal tract in the liver
E: invasion in the spleen
F: E, CK19

Aguirre et al 2003
Activation of $Kras^{G12D}$ and inactivation of $Ink4a/Arf$ in pancreatic stem cells
Activation of *Kras*\(^{G12D}\) and inactivation of *Ink4a/Arf* in pancreatic stem cells

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- wild-type p16, p53, and DPC4 2

Rozenblum E et al 1997
Activation of $Kras^{G12D}$ and $Tp53^{R172H}$ in pancreatic stem cells

(Ptf1α = p48)
Acceleration of pancreatic cancer development following activation of $Kras^{G12D}$ and $Tp53^{R172H}$ in pancreatic stem cells

Hingorani et al 2005
PDA following activation of $Kras^{G12D}$ and $Tp53^{R172H}$ in pancreatic stem cells

I: PanIN-1a
H: PDA (4.5 months)
I: liver-Met
J: ~ CK19
K: lung-Met
L: ~ CK19
M: PDA (3 months)
diff / undiff
N: ~ Alcian blue (mucin)
O: ~ CK19

Hingorani et al 2005
LOH of wild-type *Tp53* in PDA

C to H: no expression of p53 in PanIN, strong expression in PDA and liver metastasis (H)

Hingorani et al 2005
Centrosome amplification in pancreatic carcinoma cell lines

K-RAS\(^{G12D}\), Tp53\(^{R172H}\)

<table>
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<tr>
<th>cell lines:</th>
<th>primary PDA</th>
<th>liver mets</th>
<th>primary PDA</th>
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<tbody>
<tr>
<td>A and C: &gt; 2 centromers</td>
<td>E: 2 centromers</td>
<td>B and D: abnormal mitotic spindles</td>
<td>F: normal spindles</td>
</tr>
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Hingorani et al 2005
Non-reciprocal translocations in cell lines of \textit{Kras}^{G12D}, \textit{Tp53}^{R172H} pancreatic tumors

SKY-analysis
A to C and D to F: non-reciprocal translocations

FISH-analysis
 telomeric signal
G: primary duct cells
H and I: pancreatic tumor cell line

Hingorani et al 2005
Chromosomal instability (CIN) in PDA following activation of $Kras^{G12D}$ and $Tp53^{R172H}$ in pancreatic stem cells

G to I: abundant anaphases  
J: abnormal anaphase  
K and L: abnormal mitotic figures  
M and N: nuclear bridges (cell lines)

Hingorani et al 2005
Retained expression of $p16^{\text{Ink4a}}$ and Smad4(Dpc4) in cell lines of PDA

Hingorani et al 2005
Activation of $Kras^{G12D}$ and $Tp53^{R172H}$ in pancreatic stem cells

organ-specification → stem cell

PDX-1, Ptf1

PanIN-1-3
ductal pancreatic carcinoma
typical (macro)metastasis

$Kras^{G12D}$ $Tp53^{R172H}$

chromosomal instability
wildtype p16
wildtype Smad4

(Ptf1α = p48)
How does pancreatic cancer develop?

birth

start of disease

neoplasia

invasion

metastasis

Kras$^{G12D}$

Tp53$^{R172H/-}$
Inactivation of tumor suppressors alters the phenotype of \textit{K-ras}^{G12D}
How does pancreatic cancer develop?

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TGF-α / EGFR / ErbB2 / RAS - signaling

- **TGF-α** binds to its receptor, activating the EGFR/ErbB2 complex.
- EGFR/ErbB2 phosphorylate downstream targets, activating PI3K, SOS, MEK, and others.
- Sos1 activates RAS, leading to Raf activation and MEK-ERK pathway activation.
- MEK phosphorylates Erk1/2, promoting cell proliferation.
- AKT is activated downstream of PI3K, inhibiting apoptosis through Akt-mediated phosphorylation of BAD and caspase 9, and Bax/Bcl-2 regulation.
- IKK is activated, leading to IκBα degradation, increasing nuclear translocation of NF-κB.
- mTOR is activated, promoting cell growth and survival.
- PDK1 acts as a downstream kinase in the PI3K pathway, phosphorylating AKT.
- RAS activation leads to nuclear translocation and transcriptional activation of 50 and 65 gene products, among others.
Expression of \textit{Kras}^{G_{12D}} in pancreatic stem cells and of TGF\(\alpha\) in elastase positive cells

\begin{center}
\begin{tikzpicture}
\node (organ) at (0,0) [circle,draw,fill=white] {organ-specification};
\node (stem) at (2,0) [circle,draw,fill=red] {stem cell};
\node (kras) at (4,0) [circle,draw,fill=red] {\textit{Kras}^{G_{12D}}};
\node (progenitor) at (6,0) [circle,draw,fill=gray] {progenitor cell};
\node (progenitor2) at (6,2) [circle,draw,fill=gray] {PDX-1};
\node (progenitor3) at (6,-2) [circle,draw,fill=gray] {Ptf1};
\node (cancer) at (8,0) [circle,draw,fill=black] {ductal pancreatic cancer cell};
\node (cancer2) at (8,2) [circle,draw,fill=black] {PDX-1};
\node (cancer3) at (8,-2) [circle,draw,fill=black] {Ptf1};
\node (elastase) at (6,-4) [circle,draw,fill=red] {Ela-TGF\(\alpha\)};
\draw [->] (organ) -- (stem);
\draw [->] (stem) -- (kras);
\draw [->] (kras) -- (progenitor);
\draw [->] (progenitor) -- (cancer);
\draw [->] (progenitor) -- (elastase);
\end{tikzpicture}
\end{center}

(Ptf1\(\alpha\) = p48)
Expression of $Kras^{G12D}$ in pancreatic stem cells and of TGF$\alpha$ in elastase positive cells

Siveke et al unpublished
TGF\(\alpha\) expression increases Ras activity

\[
\begin{array}{c}
\text{IP} & \text{IB} \\
\text{RBD Ras} & \text{- Ras} \\
\text{wild-type} & \text{TGF\(\alpha\)} & \text{Kras} \, G12D & \text{Kras} \, G12D + \text{TGF\(\alpha\)}
\end{array}
\]
Expression of \textit{Kras}^{G12D} in pancreatic stem cells and of TGF\(\alpha\) in elastase positive cells
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Expression of $Kras^{G12D}$ in pancreatic stem cells and of TGF$\alpha$ in elastase positive cells

organ-specification  \rightarrow \text{stem cell} \rightarrow PDX-1, Ptf1 \rightarrow Kras^{G12D} \rightarrow \text{progenitor cell} \rightarrow PDX-1, Ptf1 \rightarrow \text{PanIN-1-3 ductal pancreatic carcinoma IPMT} \rightarrow \text{Ela-TGF}\alpha
IKK / $I\kappa B\alpha$ / NF-$\kappa B$ - signaling
Activation of $Kras^{G12D}$ and inactivation of $rela$ in pancreatic stem cells

(Ptf1a = p48)
Inactivation of *rela* does not alter Ras activity
H&E
1 mon

Kras$^{G12D/+}$ Ptfa$^{Cre/+}$

Kras$^{G12D/+}$ rela$^{F/F}$ Ptfa$^{Cre/+}$

100x

50x

100x

50x
H&E
2.25 mon

\[ \text{Kras}^{G12D/+} \text{Ptfa Cre/+} \]

\[ \text{Kras}^{G12D/+} \text{rela F/F Ptfa Cre/+} \]
H&E
4.5 mon

Kras^{G12D/+} Ptfa^{Cre/+}

Kras^{G12D/+ rela F/F Ptfa^{Cre/+}}
Trichrom-stain

Kras$^{G12D/+}$ Ptfa$^{Cre/+}$

Kras$^{G12D/+}$ rela$^{F/F}$ Ptfa$^{Cre/+}$

2.25 mon

4.5 mon
4.5 mon

Kras<sup>G12D/+ Ptfα Cre/+</sup>  

Kras<sup>G12D/+ rela F/F Ptfα Cre/+</sup>

CK-19

Amylase
MUC-5

Kras\textsuperscript{G12D/+} Ptfa\textsuperscript{Cre/+}

Kras\textsuperscript{G12D/+} rela\textsuperscript{F/F} Ptfa\textsuperscript{Cre/+}

2.25 Mon

4.5 Mon
Activation of $Kras^{G12D}$ and inactivation of $rela$ in pancreatic stem cells

- organ-specification
- stem cell
- PDX-1
- Ptf1
- $Kras^{G12D}$
- $rela^{-/-}$
- (Ptf1a = p48)

- progression of PanIN-1-3
- loss of acinar cells
- increased number of CK19 positive cells
- increased desmoplastic reaction
How does pancreatic cancer develop?
CXCL12 - CXCR4 signaling induces metastasis
CXCL12 - CXCR4 signaling induces metastasis

peripheral blood

blood vessel wall

stroma cells

CXCL12
CXCR4

neutrophils
tumor cells

metastasis

CXCL12 – CXCR4 signaling induces metastasis
Detection of tumor cell growth in vivo

day 5 p.i.

day 7 p.i.

day 10 p.i.

day 15 p.i.
CXCR4 overexpression induces liver and lung metastasis
CXCR4 overexpression induces liver and lung metastasis

<table>
<thead>
<tr>
<th>cell line</th>
<th>Tumorigenicity</th>
<th>Frequency (metastasis/total)</th>
<th>Lung (metastasis/total)</th>
<th>Liver (metastasis/total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TD-2 fLuc</td>
<td>14/14</td>
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**CXCR4 overexpression induces liver and lung metastasis**

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<td>TD-2 fLuc CXCR4 + AMD 3100</td>
<td>7/7</td>
<td>1/7</td>
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How does pancreatic cancer develop?

- Start of disease
- Neoplasia
- Invasion
- Metastasis
- Birth
- CXCR4
How does pancreatic cancer develop?

- environment
  - start of disease
    - PanINs
    - invasion
    - metastasis

- genetic predisposition
  - birth
    - \textit{Kras}^{G12D}
    - \textit{Tp53}^{R172H/-}
    - \textit{Ink4a/Arf}^-/
  - \textit{CXCR4}
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