Pancreatic Cancer: Current Understanding and Future Challenges

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Pancreatic Cancer Unit
Laboratory of Human Carcinogenesis
Cancer incidence and mortality

Pancreatic Cancer Incidence and Mortality

<table>
<thead>
<tr>
<th>Estimated Deaths</th>
<th>Siegel R et. al., CA Cancer J Clin, 65, 2015</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>Lung &amp; bronchus</td>
</tr>
<tr>
<td>Prostate</td>
<td>Breast</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>Colon &amp; rectum</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Pancreas</td>
</tr>
<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>Ovary</td>
</tr>
<tr>
<td>Leukemia</td>
<td>Leukemia</td>
</tr>
<tr>
<td>Esophagus</td>
<td>Uterine corpus</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>Non-Hodgkin lymphoma</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>Liver &amp; intrahepatic bile duct</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>Brain &amp; other nervous system</td>
</tr>
<tr>
<td>All Sites</td>
<td>312,150</td>
</tr>
<tr>
<td></td>
<td>277,280</td>
</tr>
<tr>
<td></td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>100%</td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>86,380</td>
</tr>
<tr>
<td>Prostate</td>
<td>27,540</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>26,100</td>
</tr>
<tr>
<td>Pancreas</td>
<td>20,710</td>
</tr>
<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>17,030</td>
</tr>
<tr>
<td>Leukemia</td>
<td>14,210</td>
</tr>
<tr>
<td>Esophagus</td>
<td>12,600</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>11,510</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>11,480</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>9,070</td>
</tr>
</tbody>
</table>

- 4th Leading Cause of Cancer Deaths in the United States.
- Median Survival < 6 Months.
- No Effective Treatment.
Pancreatic cancer deaths are increasing

Pancreatic Cancer: Second Leading Cause of Cancer-related Death by 2030

Rahib, L., et. al., Cancer Res., 74, 2913-21, 2014
### Risk Factors and Inherited Syndromes

<table>
<thead>
<tr>
<th>Variable</th>
<th>Approximate Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk factor</td>
<td></td>
</tr>
<tr>
<td>Smoking³</td>
<td>2–3</td>
</tr>
<tr>
<td>Long-standing diabetes mellitus⁴</td>
<td>2</td>
</tr>
<tr>
<td>Nonhereditary and chronic pancreatitis⁵</td>
<td>2–6</td>
</tr>
<tr>
<td>Obesity, inactivity, or both⁶</td>
<td>2</td>
</tr>
<tr>
<td>Non-O blood group⁷</td>
<td>1–2</td>
</tr>
<tr>
<td>Genetic syndrome and associated gene or genes — %</td>
<td></td>
</tr>
<tr>
<td>Hereditary pancreatitis (PRSS1, SPINK1)⁸</td>
<td>50</td>
</tr>
<tr>
<td>Familial atypical multiple mole and melanoma syndrome (p16)⁹</td>
<td>10–20</td>
</tr>
<tr>
<td>Hereditary breast and ovarian cancer syndromes (BRCA1, BRCA2, PALB2)¹⁰,¹¹</td>
<td>1–2</td>
</tr>
<tr>
<td>Peutz–Jeghers syndrome (STK11 [LKB1])¹²</td>
<td>30–40</td>
</tr>
<tr>
<td>Hereditary nonpolyposis colon cancer (Lynch syndrome) (MLH1, MSH2, MSH6)¹³</td>
<td>4</td>
</tr>
<tr>
<td>Ataxia–telangiectasia (ATM)¹⁴</td>
<td>Unknown</td>
</tr>
<tr>
<td>Li–Fraumeni syndrome (P53)¹⁵</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

* Values associated with risk factors are expressed as relative risks, and values associated with genetic syndromes are expressed as lifetime risks, as compared with the risk in the general population.

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Ryan, Hong and Bardeesy, NEJM, 371, 2014
Pancreatic cancer patient treatment

Disappointing Progress in the Treatment of Pancreatic Cancer

  - Gemcitabine vs 5-FU (Log-Rank Test p=0.0025)
  - Median survival: Gemcitabine n=63, 5-FU n=63, 5.65 vs 4.41 months

- **Moore et. al., J. Clin. Oncol. 25, 2007**
  - Gemcitabine + Erlotinib
  - HR = 0.61 (95% CI 0.67 to 0.98, P = .03)
  - Erlotinib vs Placebo (n=261): Median = 6.96 months, 1-year survival = 23%
  - Placebo (n=260): Median = 6.56 months, 1-year survival = 17%

- **Conroy et. al., NEJM, 36, 2011**
  - Folfirinox
  - Hazard ratio, 0.57 (95% CI, 0.45–0.73), P < 0.001 by stratified log-rank test
  - Median survival: FOLFIRINOX 11.1 months, Gemcitabine 6.8 months

- **Von Hoff, D.D. et. al, NEJM, 369, Oct, 2013**
  - Gemcitabine + nab-Paclitaxel
  - Overall survival
  - Hazard ratio for death, 0.72 (95% CI, 0.62–0.83), P = 0.001 by stratified log-rank test
  - Median survival: nab-Paclitaxel-Gemcitabine 8.5 months, Gemcitabine 6.7 months

  - Nanoliposomal irinotecan+fluorouracil+folinic acid
  - Hazard ratio, 0.67 (95% CI, 0.49–0.91), P = 0.02 (unstratified log-rank)
  - Median survival: Nanoliposomal irinotecan 4.2 months, Fluorouracil and folinic acid 6.1 months
Resected pancreatic cancer

Improved Survival in Resected Pancreatic Cancer Cases

Late Diagnosis

Dismal Outcome (Median Survival < 6 mo) (18-20%)

Resectable Cases (Median Survival < 2 yrs) (~12%)

5 yr Survival

Molecular Differences in Tumors Determine Patient Outcome?
Tumor biology

Understanding Pancreatic Tumor Biology is Key to Improving Disease Outcome

Pancreatic Cancer
(Median Survival < 6 mo)

Late Diagnosis

Early Detection

- Biomarkers-Precancerous Lesions

Tumor Biology

Better Outcome

Effective Therapy

- Novel Targets
- Molecular Subtypes
- Treatment Selection
- Drug Delivery

Poor Therapeutic Response
Pancreatic carcinogenesis

Progression Model of Pancreatic Carcinogenesis

Pancreatic Intraepithelial Neoplasia

Normal → PanIN-1 → PanIN-2 → PanIN-3 → Cancer

Telomere Shortening

- KRAS2
- CDKN2A
- TP53
- SMAD4
Desmoplastic stroma

Prominent, Desmoplastic Stroma in Pancreatic Cancer

Glypican-1 Positive Circulating Exosomes as a Biomarker for PDAC

Glycan-1 positive exosomes

Glypican-1 Positive Circulating Exosomes Predicts Prognosis in Resected PDAC Patients

Pancreatic cancer and tumor heterogeneity

Tumor heterogeneity and molecular subtypes.
Heterogeneity

Pancreatic Cancer is Highly Heterogenous

Molecular subtypes

Are There Different Molecular Subtypes of PDAC?

Collison et. al., Nat. Med., 17, 2011
Chromosomal structure

Variations in Chromosomal Structure and PDAC Subtypes

Stroma specific subtypes

Stroma-Specific Subtypes in Pancreatic Cancer

Moffitt et. al., Nature Genetics, 2015
Four PDAC subtypes

Gene Expression Analysis Identified 4 PDAC Subtypes


ADEX: Aberrantly differentiated exocrine and endocrine
Metabolic subtypes

Metabolic Subtypes in Pancreatic Cancer

Daemen et. al., PNAS, 2015
Metabolic Reprogramming in Pancreatic Cancer

Sousa and Kimmelman, Carcinogenesis, 35, 2014
Dessert

Dessert Anyone?

RESEARCH HIGHLIGHTS

TUMOUR METABOLISM

Feeding your friends

A characteristic feature of pancreatic ductal adenocarcinoma (PDAC) is a strong stromal reaction that is shaped by the activity of pancreatic stellate cells (PSCs). The resultant fibrosis impedes the tumour's access to a blood supply, creating an extremely hypoxic, nutrient-poor environment. This forces the mitochondria and not the cytosol. As a result, carbon derived from consumed alanine feed the tricarboxylic acid (TCA) cycle to increase oxygen consumption while not affecting glycolysis. Indeed, citrate with this, inhibition of autophagy in PSCs could reduce the growth of...
Pancreatic stellate cells support tumor metabolism

- Pancreatic stellate cells
  - Supports proliferation (in low-nutrient environment)
  - Releases free amino acids

- Cancer cells
  - Fuels TCA cycle
  - Supports lipid and NEAAs biosynthesis
  - Shunts glucose to Ser/Gly biosynthesis
  - Increases autophagy

Therapeutic resistance

Complex Stromal Networks Supporting Pancreatic Cancer Progression and Therapeutic Resistance

Stromnes, I.M., et. al., Carcinogenesis, 35, 2014
Targeting cancer

Treatment Strategies to Improve Disease Outcome

Drug Delivery
and
Effectiveness of Systemic Therapy

Targeting Stroma
Mouse models

Pancreatic Cancer Mouse Model (KPC)

*LSL-Kras-G12D  X  p53 LSL R172H  X Pdx-Cre 1

↓

Pancreatic Ductal Adenocarcinoma (PDAC)

(Median Survival = 4-5 months)

*Hingorani, S. et. al., Cancer Cell, 2005
Hedgehog signaling

Inhibition of Hedgehog Signaling Depleted Stroma, Enhanced Drug Delivery and Improved Survival in Mice

Olive KP et. al., Science, 324, 2009
Stroma targeting

Enzymatic Targeting of Stroma Enhances Therapeutic Response

Proenzano et. al., Cancer Cell, 21, 2012
Therapeutic response

Enzymatic Targeting of Stroma Enhances Therapeutic Response

Proenzzano et. al., Cancer Cell, 21, 2012
Anti-stromal tissue

Two Faces of Anti-Stromal Therapy

Stromal-targeting may not (always) have beneficial therapeutic response
Sonic Hedgehog

Sonic Hedgehog as a Tumor Suppressor in PDAC

Genetically Engineered Mouse Model

C

Percent Survival

PKCY (n=26)
Shh-PKCY (n=23)
p=0.0049, Log Rank

Time (months)

D

Survival from Palpation of Tumor (days)

PKCY
ShhPKCY
p < 0.001

E

Macrometastasis (Percent)

PKCY
ShhPKCY
p = 0.039

F

Number / 80 hpf

A = Acinar to Ductal Metaplasia
1 = PanIN1
2 = PanIN2
3 = PanIN3

Rhim AD et. al., Cancer Cell, 25, 2014
Myofibroblast Depletion Enhances PDAC

Ozdemir, BC et. al., Cancer Cell, 25, 2015
Myofibroblast depletion

Myofibroblast Depletion Reduces Overall Survival

GCV = ganciclovir (Depletes Myofibroblasts in PKT;αSMA-tk+ Mice)
Tumor-Stromal interaction is complex and therapeutic approaches targeting stroma needs caution and may require new molecular taxonomy in pancreatic cancer
Mesothelin and Immunotherapy

Mesothelin as a Target for Immunotherapy

Pastan and Hassan, Cancer Res., 2014
Mesothelin targeted T cells

Mesothelin Targeted T Cells Lyse Tumor Cells and Increase Survival in KPC Mouse Model of PDAC

Stromnes et al., Cancer Cell, 28, 2015
Organoid: A highly promising model for PDAC

Boj et. al., Cell, 160, 2015
Hwang et. al., J. Pathology, 238, 2016
Inflammation and Pancreatic Cancer

Chronic Pancreatitis

Desmoplastic Stromal Formation
Inflammatory Cytokines

NF-κB Signaling
COX2, NOS2
Pancreatic Cancer Development

Inflammatory Changes During Development and Progression of Pancreatic Cancer


CP= Chronic Pancreatitis
PSC= Pancreatic Stellate Cells
ECM= Extracellular Matrix
Inflammation and Pancreatic Cancer

Inflammation Enhances and Maintains a Pathologic Level of Oncogenic KRAS in Pancreatic Cancer

Daniluk et. al., JCI, 2012
MIF and Cancer

Macrophage Migration Inhibitory Factor (MIF)

- Increased Expression in Tumors
  - NF-κB
  - ERK1/2
  - Akt
  - COX2
  - p53
  - Rb/E2F
  - NOS2

- Proinflammatory Cytokine
- Expressed in Epithelial and inflammatory Cells

Immune/Inflammatory Response
MIF, Inflammation and Cancer

At the Crossroads of Inflammation and Tumorigenesis
By Carlos Cordon-Cardo* and Carol Prives†

From the *Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, New York 10021; and the †Department of Biological Sciences, Columbia University, New York, New York 10027

Macrophage Migration Inhibitory Factor MIF Interferes with the Rb-E2F Pathway
Oleksii Petrenko* and Ute M. Moll*

Macrophage Migration Inhibitory Factor: A Probable Link between Inflammation and Cancer
Richard Bucala1,* and Seamas C. Donnelly2,*
MIF Contributes to Pancreatic Cancer Progression and Predicts Disease Outcome.
MIF and PDAC

Increased expression of MIF in tumors from pancreatic ductal adenocarcinoma cases
MIF expression and HDAC survival

A higher expression of MIF is associated with poor survival in human PDAC

Human Pancreatic Carcinoma Cases

<table>
<thead>
<tr>
<th>Variables (comparison/referent)</th>
<th>Univariable Analysis</th>
<th>Multivariable Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95%CI)</td>
<td>P</td>
</tr>
<tr>
<td>MIF (High/Low)</td>
<td>2.21 (1.16-4.22)</td>
<td>0.016</td>
</tr>
<tr>
<td>Grading (G3-4/G1-2)</td>
<td>1.86 (1.01-3.45)</td>
<td>0.048</td>
</tr>
<tr>
<td>Resection margin (R1/R0)</td>
<td>1.53 (0.82-2.83)</td>
<td>0.178</td>
</tr>
<tr>
<td>Stage (IIIB-III/I-IIA)</td>
<td>1.62 (0.79-3.36)</td>
<td>0.191</td>
</tr>
</tbody>
</table>

MIF expression and poor PDAC survival

A higher expression of MIF is associated with poor survival in human PDAC

Validation in Independent Cohorts

Human PDAC Cases

Validation Cohort 1
P = 0.007
Low MIF (N=11)
High MIF (N=11)

Validation Cohort 2
P = 0.020
Low MIF (N=13)
High MIF (N=13)
MIF accelerates tumor growth

MIF accelerates tumor growth and metastasis
In orthotopic xenografts in mice
MIF and gene expression

MIF Induces a marked change in global gene expression profile including EMT-related genes in orthotopic tumors

- MIF over-expressing tumors are poorly differentiated.
- MIF induces a change in global gene expression profile.
- MIF over-expressing tumors showed expression of EMT-marker genes.
Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver

Bruno Costa-Silva¹, Nicole M. Aiello⁷, Allyson J. Ocean⁹, Swarnima Singh¹, Haiying Zhang¹, Basant Kumar Thakur¹⁴, Annette Becker¹, Ayuko Hoshino¹, Milica Tešić Markš, Henrik Molina³, Jenny Xiang⁶, Tuoh Zhang⁶, Till-Martin Theilen¹, Guillermo García-Santos¹, Caitlin Williams¹, Yonathan Ararsö¹, Yujie Huang⁴, Gonçalo Rodrigues¹⁷, Tang-Long Shen⁶, Knut Jørgen Labori⁹, Inger Marie Bovitz Lothe¹⁰,¹¹, Elin H. Kure¹¹, Jonathan Hernandez¹², Alexandre Doussot¹³, Sayla H. Ebbesen¹, Paul M. Grandgenett¹³, Michael A. Hollingsworth¹³, Maneesh Jain¹⁴, Kavita Mallya¹⁴, Surinder K. Batra¹⁴, William R. Jarnagin¹², Robert E. Schwartz¹⁵, Irina Matei³, Héctor Peinado¹,¹⁶, Ben Z. Stanger²,¹⁹, Jacqueline Bromberg¹⁷,¹⁹ and David Lyden¹,¹⁸,¹⁹

Pancreatic ductal adenocarcinomas (PDACs) are highly metastatic with poor prognosis, mainly due to delayed detection. We hypothesized that intercellular communication is critical for metastatic progression. Here, we show that PDAC-derived exosomes induce liver pre-metastatic niche formation in naive mice and consequently increase liver metastatic burden. Uptake of PDAC-derived exosomes by Kupffer cells caused transforming growth factor β secretion and upregulation of fibronectin production by hepatic stellate cells. This fibrotic microenvironment enhanced recruitment of bone marrow-derived macrophages. We found that macrophage migration inhibitory factor (MIF) was highly expressed in PDAC-derived exosomes, and its blockade prevented liver pre-metastatic niche formation and metastasis. Compared with patients whose pancreatic tumours did not progress, MIF was markedly higher in exosomes from stage I PDAC patients who later developed liver metastasis. These findings suggest that exosomal MIF primes the liver for metastasis and may be a prognostic marker for the development of PDAC liver metastasis.
MIF-induced disease

MIF-induced disease aggressiveness in pancreatic cancer

Human PDAC Cases

H/E

High MIF

Low MIF

OMICS

miRNA

mRNA

Questions

- Molecular Distinctions
- Mechanistic and Functional Role of MIF in Tumor Progression

High MIF

Low MIF
miRNA profiling

miRNA profiling of MIF-high and MIF-low tumors

- Hypothesis: MIF regulates miRNAs associated with tumor progression and disease aggressiveness in patient with pancreatic cancer

Differentially Expressed miRNAs
MIF-high vs MIF-low

53 Differentially Expressed miRNAs
MIF-high Vs. MIF-Low
(p<0.05)

miRNAs Associated with Survival
(Kaplan-Meier Analysis)

5 miRNAs Associated with Survival
- miR-301b
- miR-15b
- miR-10b
- miR-93
- miR-590-5p

Validation
(Multiple Cohorts of PDAC)
MIF axis in Pancreatic Cancer

MIF/miR-301b/NR3C2 Axis in Pancreatic Cancer
MIF/mir-301b/NR3C2 Signaling is a Potential Therapeutic Target in Pancreatic Cancer
Pancreatic Tumors Express MIF

Pancreatic tumors in KPC mice express a high level of MIF

MIF deletion in genetically engineered mouse model of pancreatic cancer
MIF deficiency enhances survival

MIF-deficiency enhanced survival and reduced metastasis in KPC mice

KPC Mouse Model

**Survival**

- MKPC (N=48)
- KPC (N=31)

**Metastasis**

- % of Mice with Metastasis
  - KPC (N=31)
  - MKPC (N=48)

- % of Mice with Liver Metastasis
  - KPC (N=31)
  - MKPC (N=48)

MKPC= MIF-deficient KPC mice
MIF deficiency reduces EMT

MIF-deficiency reduced EMT in KPC mice
MIF deficiency increases NR3C2 expression

MIF-deficiency decreases miR-301b and increases NR3C2 expression
MIF inhibition strategies

Strategies for MIF inhibition

- HSP90 Inhibition
- Soluble MIF Receptor Antagonist
- Small Molecule MIF-Antagonists
- Anti-MIF Receptor Antibodies
- Anti-MIF Antibodies (NCT01765790 Phase 1)

*Richard Bucala, Yale Univ. Sch. of Med.*
A higher MIF expression is associated with poorer outcome in PDAC patients.

MIF enhances growth and metastasis of tumor xenografts in mice.

MIF-driven signaling inhibits NR3C2 by upregulating miR-301b.

NR3C2 is a negative regulator of EMT.

MIF-deficiency increased survival and reduced metastasis in KPC mice.

MIF/mir-301b/NR3C2 signaling is a potential therapeutic target.
Understanding pancreatic tumor biology

Understanding Pancreatic Tumor Biology is Key to Improving Disease Outcome

Pancreatic Cancer

(Median Survival < 6 mo)

Late Diagnosis

Poor Therapeutic Response

Early Detection

Biomarkers-Precancerous Lesions

Tumor Biology

Better Outcome

Effective Therapy

• Novel Targets
• Molecular Subtypes
• Treatment Selection
• Drug Delivery