Pancreatic Cancer
Where Stemness Drives Malignancy
Pancreatic Cancer: US Impact

- 12th most common cancer\(^3\)
- 53,000 diagnosed annually\(^{1,2}\)
- 3.1% of all new cancer cases\(^{1,2}\)
- Over 40,000 deaths per year\(^{1,2}\)
- 7% of all cancer deaths\(^{1,2}\)
- 7.7% 5-year survival\(^{1,2}\)

3rd leading cause of cancer death\(^3\)

Pancreatic Cancer: Prognosis is Poor

5-year Survival Based on Disease Stage at Diagnosis

<table>
<thead>
<tr>
<th>Stage at Diagnosis</th>
<th>Localized</th>
<th>Regional</th>
<th>Metastatic</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival</td>
<td>29.3%</td>
<td>11.1%</td>
<td>2.6%</td>
<td>7.7%</td>
</tr>
</tbody>
</table>

The majority of patients are diagnosed with metastatic disease due to few indicative symptoms in early stages and a lack of effective screening.

Pancreatic Cancer: Treatment Algorithm and Approvals

Good Performance Status

- Surgery
- First Line: Combination chemotherapy and/or chemoradiation

Poor Performance Status

Second Line: Combination chemotherapy or chemoradiation

Palliative Care

1995

- Gemcitabine
- 18 year gap in drug approvals

2013

- nab-Paclitaxel
- Irinotecan liposome

Median overall survival still < 12 months despite new treatments

Pancreatic Cancer: Solving the Puzzle

- 90% of pancreatic cancers are diagnosed after they have spread\(^1\)
- Pancreatic tumor cells are frequently resistant to chemotherapy or become resistant during chemotherapy\(^2\)
- Recurrence is typical, even after successful resection\(^3\)

To find potential causes of pancreatic cancer, tumors were analyzed to identify upregulated signaling pathways and genetic mutations in cancers

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Pancreatic Cancer: Upregulated Pathways

Analysis of clinical pancreatic cancer samples identified several pathways that were commonly upregulated.

These pathways are typically active in Cancer Stem Cells or in cells that have developed "Stemness" characteristics.

STAT3, signal transducer and activator of transcription 3; TGF-β, transforming growth factor beta.

Cancer Stem Cells: Characteristics

- Ability to self-renew and differentiate
- Convey tumorigenicity
- Show innate resistance to multiple conventional therapies
- Play a key role in metastasis
- Have dysregulated stemness signaling pathways

*Cells with these properties have “Stemness” characteristics

*Bulk tumor cells.

Stemness Characteristics Have Multiple Outcomes

- **Cancer Stem Cells** are a subset of cells existing within a tumor with high tumor-initiating capabilities.

- **Tumorigenesis** due to unregulated self-renewal and differentiation.

- **Metastasis** due to EMT-mediated increase in migration and invasiveness.

- **Resistance** due to activation of multiple resistance-mediating genes.

- **Acquisition of transient stemness** increases ability to metastasize.

- **Innate resistance** to conventional chemo- and radiotherapies may underlie tumor recurrence.

**EMT**, epithelial-mesenchymal transition.
Key Stemness Signaling Pathways Drive Malignancy in CSCs

- Dysregulation or activation of these pathways can result in stemness characteristics
  - Self-renewal
  - Differentiation
  - Migration
  - Tumorigenicity
  - Innate resistance

Several of these pathways are upregulated in pancreatic cancers

CSC, cancer stem cell; JAK, Janus kinase; STAT, signal transducer and activator of transcription.

Pancreatic Cancer Stem Cell Discovery

- Human PDAC cells from 8 primary and 2 metastatic tumors were analyzed.
- Highly tumorigenic subpopulation (0.2-0.8% pancreatic cells) had 100-fold increased tumorigenic potential compared with bulk tumor cells.

Heterogeneous tumor

PCSC

Self-renewal

100-fold tumorigenic potential vs non-Stem Cancer Cells

Differentiation

Non-Stem Cancer Cells*

Pancreatic CSCs showed CD44+ CD24+ ESA+

*Bulk tumor cells.

CSC, cancer stem cell; PCSC, pancreatic cancer stem cell; PDAC, pancreatic adenocarcinoma.

Cancer Stem Cells Can be Identified by Stemness Markers

- Many CSCs, but not all, can be identified by distinctive cancer stemness markers\(^1,2\)
- CSC marker profiles differ between tumors and can change over time\(^1,3\)
- Marker expression may be influenced by the pathways that are active\(^1,3\)

### Cancer Stemness Markers\(^4-6\)

<table>
<thead>
<tr>
<th>ALDH(^+), ALDH1(^{high})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\alpha2\beta4)^{high}, A2B5(^+), ABCG2(^{high})</td>
</tr>
<tr>
<td>BCRP1(^+), BMI1(^+)</td>
</tr>
<tr>
<td>CD24(^+), CD24(^{-})(\text{low}), CD34(^+), CD38(^-), CD44(^+), CD44v6, CD49f(^+), CD54, CD71(^-), CD90(^+), CD90(^-), CD117(^-), CD123(^+), CD133(^+), CD138(^-), CD166(^+), claudin7</td>
</tr>
<tr>
<td>CXCR4(^+), CK20(^+), CEA(^+), c-Met</td>
</tr>
<tr>
<td>ESA(^+), EpCAM(^+)</td>
</tr>
<tr>
<td>Lineage(^-), LGR5(^+)</td>
</tr>
<tr>
<td>SSEA(^+), Tspan8, HLA-DR(^-), YAP1(^+)</td>
</tr>
</tbody>
</table>

The most common stemness markers identified on pancreatic CSCs are CD44\(^+\) CD24\(^+\) ESA\(^+\)\(^5\)

ALDH, aldehyde dehydrogenase; CSC, cancer stem cell.

Cancer Stem Cells and Stemness Activity Predicted Poor Survival in Patients with Pancreatic Cancer

- PCSCs identified via biomarkers, and cells with activated stemness pathways, were associated with lower overall survival\(^1,2\)

*Log-rank test showed correlation between Nanog and survival was significant.
ALDH, aldehyde dehydrogenase; PCSC, pancreatic cancer stem cell.
Stemness Pathways in Pancreatic Cancer Progression
Stemness Pathways Activated in Stressed Pancreas May Initiate Tumor Formation

*Stress includes damage to the pancreatic tissue that requires tissue repair, including disease, inflammatory conditions, or burden from food.

CSC, cancer stem cell; STAT3, signal transducer and activator of transcription 3.

STAT3 May be a Key Factor in Initiation of Pancreatic Cancer

↑K-ras gain-of-function mutations in pancreatic cells

IL-6 released as inflammatory response

PanINs

Pancreatic cancer patients have high levels of serum IL-6 and activated STAT3

Pancreatitis

Inflammation in both pancreatic cells and the microenvironment activate STAT3, initiating neoplasia

Pancreatitis is a known risk factor for pancreatic ductal adenocarcinoma

IL-6, interleukin 6; PanIN, pancreatic intraepithelial neoplasia; STAT3, signal transducer and activator of transcription 3.

STAT3: Positive and Negative Feedback Loops Drive Tumorigenicity in Pancreatic Cancer

Positive inflammatory response feedback drives tumorigenesis\(^1,2\)

\[ \uparrow \text{NF-κB} \quad \uparrow \text{MAP Kinase} \]

Tumor microenvironment

\[ \uparrow \text{TLR7} \quad \uparrow \text{Notch} \]

\[ \downarrow \text{miRNA} \quad \downarrow \text{SOCS} \]

Pancreatic cancer cell proliferation

IL-6, interleukin 6; miRNA, microRNA; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; SOCS, suppressor of cytokine signaling; STAT3, signal transducer and activator of transcription 3; TLR7, toll-like receptor 7.

Hedgehog: Multiple Roles Drive Pancreatic Cancer Progression

Tumor initiation

Pancreatic duct glands display gastrointestinal metaplasia phenotype

K-ras "crosstalk"

$\uparrow$ NF-κB

$\uparrow$ GLI-1 $\downarrow$ PTCH1

PCSC self-renewal & differentiation

Sonic hedgehog signaling

$\uparrow$ VEGF

Angiogenesis

Promotes stromal growth

NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PCSC, pancreatic cancer stem cell; VEGF, vascular endothelial growth factor.


The hedgehog pathway contributes to the tumorigenicity, maintenance and progression of pancreatic cancer.
Pancreatic CSCs May Influence Adjacent non-Stem Cancer Cells

- PCSCs can signal adjacent non-stem cancer cells to de-differentiate
- Direct cell-cell contact could activate signal cascades via cell surface biomarkers
- Genetic and epigenetic information could be transferred to adjacent cells

CSC, cancer stem cell; EMT, epithelial-mesenchymal transition; PCSC, pancreatic cancer stem cell.
Pancreatic Cancer Cells Induce Changes in Tumor Microenvironment

Direct contact between PDAC cell and CAF

DNA methylation → ↓SOCS1 → STAT3 activated in absence of SOCS1

Transcribes cytokines and growth factors that are released by CAFs

IGF-1, IGF-1, IL-6, OSM, TNF-α

Malignant growth and progression of tumor

Pancreatic cancer cells induce epigenetic changes in stromal cells that support growth of cancer

Patients with epigenetic suppression of SOCS1 have reduced survival rates

CAF, cancer-associated fibroblast; IGF-1, insulin-like growth factor 1; IL-6, interleukin 6; OSM, oncostatin M; PDAC, pancreatic ductal adenocarcinoma; SOCS, suppressor of cytokine signaling; STAT3, signal transducer and activator of transcription 3; TNF-α, tumor necrosis factor alpha.

Microenvironment Can Induce Progression and Metastasis in Pancreatic Cancer

- The tumor microenvironment, or stem cell niche, surrounding PCSCs can induce cancer progression\(^1,2\)

- Expression of PCSC biomarkers is influenced by tumor stroma\(^1\)

- Soluble factors secreted by stromal cells signal pathways for\(^3\)
  - Proliferation
  - Invasion
  - Tumor immunity
  - Angiogenesis

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CSC, cancer stem cell; EMT, epithelial-mesenchymal transition; G-CSC, gastric cancer stem cell; MCP-1, monocyte chemotactic protein-1; miRNA, microRNA; NO, nitric oxide; PCSC, pancreatic cancer stem cell; ROS, reactive oxygen species; SDF-1\(\alpha\), stromal-derived factor-1 alpha; TGF-\(\beta\), transforming growth factor beta; TNF-\(\alpha\), tumor necrosis factor alpha; VEGFA, vascular endothelial growth factor A.

Stemness Characteristics Drive Metastasis in Pancreatic Cancer

- Epithelial-Mesenchymal Transition (EMT) is a critical step in metastasis\(^1\)
- PCSC and EMT share pathways and phenotypes\(^2\)

Multiple markers and pathways upregulated in PCSCs drive EMT\(^2\)

Epithelial cells acquire stemness characteristics\(^1,3,4\)

\[\uparrow\text{ZEB1, Twist, Snail and Slug suppress E-cadherin}\]
\[\downarrow\text{E-cadherin causes loss of cell-cell adhesion}\]
\[\uparrow\text{MMP9, which breaks down extracellular matrix}\]
\[\uparrow\text{Increased migration and invasiveness}\]
\[\uparrow\text{Ability to self-renew and differentiate}\]

ALDH, aldehyde dehydrogenase; MMP9, matrix metalloproteinase-9; PCSC, pancreatic cancer stem cell; TGF-\(\beta\)1, transforming growth factor beta 1.

Stemness May Mediate Resistance to Therapy
Gemcitabine May Induce Resistance in Pancreatic Cancer

EGFR-STAT3 signaling increased

↑MMP7, ↑CyclinD, ↑Survivin

↑EMT, invasion, and metastases

Apoptosis blocked

PCSCs have shown high resistance to standard gemcitabine therapy

Multiple PCSC biomarkers are increased in resistant cells

↑CD24+, ↑CD44+, ↑CD133+, ↑EpCAM, ↑Oct4, ↑PDX1, ↑Nanog

Gemcitabine removed

Cell cycle arrested but no apoptosis

Faster, more aggressive tumor growth

Bulk tumor cells killed but PCSCs remain viable

Gemcitabine

EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; MMP7, matrix metalloproteinase-7; PCSC, pancreatic cancer stem cell; STAT3, signal transducer and activator of transcription 3.

Macrophages Confer Chemoresistance via STAT3 Activation in Tumor Cells

Tumor-associated Macrophages (TAMs)

Activates STAT3

↑IL-10, VEGF, TGFβ, IL-23

Increased expression of immunosuppression factors

Macrophages

STAT3

Neutrophil

Dendritic cell

Natural killer cell

Activates Wnt pathway

TAM-mediated STAT3 activation is directly related to PCSC chemoresistance

Blocks T-cell recruitment to microenvironment

TH1 immune response suppressed

IL-10, interleukin 10; IL-23, interleukin 23; PCSC, pancreatic cancer stem cell; STAT3, signal transducer and activator of transcription 3; TGFβ, transforming growth factor beta; TH1, T helper cell; VEGF, vascular endothelial growth factor.

Radioresistance Linked to Inhibition of Apoptosis

Pancreatic cancer cells may confer radiotherapy resistance through activation of cell survival and proliferation pathways.
Stemness: The Next Step in Targeting Pancreatic Cancer
Pancreatic Cancer: Solving the Puzzle

- Pancreatic cancers show an upregulation in stemness pathways\(^1\)
- PCSCs can induce stemness characteristics in non-stem cancer cells and stromal cells, driving EMT and progression\(^2\)
- Resistance to therapy appears to be mediated through stemness pathway activation in both PCSCs and non-stem cancer cells\(^3\)

Stemness pathways drive malignancy in pancreatic cancer

EMT, epithelial-mesenchymal transition; PCSC, pancreatic cancer stem cell.
Multiple Potential Targets of Cancer Stemness in Pancreatic Cancer

Stemness Signaling Pathways
- Nanog
- Wnt/β-catenin
- Hedgehog
- Notch
- JAK/STAT
- PI3K/Akt

Microenvironmental Influences on Stemness

Cancer Stemness Markers
- ALDH+, ALDH1high
- a2b1high, A2B5+, ABCG2high
- BCRP1+, BMI1+
- CD24+, CD24-low, CD34+, CD38-, CD44+, CD44v6, CD49f, CD54, CD71+, CD90+, CD90−, CD117+, CD123+, CD133+, CD138−, CD166+
- CXCR4+, CK20+, CEA+, c-Met
- ESA+, EpCAM+
- Lineage-, LGR5+
- SSEA+, HLA-DR−, YAP1+

Bold=identified on pancreatic CSCs.

ALDH, aldehyde dehydrogenase; CSC, cancer stem cell; JAK, Janus kinase; STAT, signal transducer and activator of transcription.
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