Tumor Stroma

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Outline

• Overview of stroma composition
• Focus on Cancer Associated Fibroblasts (CAFs)
  – Origins of CAFs
  – Stromal – epithelial interaction
  – Genetics and Epigenetics of CAFs
  – Prognostic value of CAFs
  – Significance of CAFs in tumor progression and metastasis
• Therapeutic implications and results
Practical questions about the cancer associated stroma

• Chicken and Egg dilemma
  – Do cancer cells induce a desmoplastic reaction or does an altered microenvironment provide a permissive milieu for tumor formation?

• Friend or Foe (or Frenemy?)
  – Does ablation or reduction of tumor stroma benefit

• Primary Therapeutic Target or Adjunct?
Components of the tumor stroma or tumor microenvironment

- Components of the stroma
  - Vascular cells
    - Endothelial cells
    - Pericytes
  - Leukocytes
    - Myeloid
    - Lymphoid
  - Neural cells
  - Extracellular Matrix
  - Mesenchymal cells
    - (Myo)fibroblasts/Cancer Associated
    - Mesenchymal stem cells
    - Fibroblasts
Tumors are “non-healing wounds”
Fibroblasts and Myofibroblasts

- The major stromal cell type in most cancers
- Slender fusiform smooth nucleus
- Cancer Associated Fibroblasts (CAFs) are often equated with activated fibroblasts or myofibroblasts
  - The cancer stroma does contain both fibroblasts and myofibroblasts
- Myofibroblasts are capable/responsible for tissue contraction and secretion of ECM
  - The hallmark is αSMA expression
A nearly ubiquitous feature of most solid cancers

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Estimated % stroma</th>
</tr>
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<tbody>
<tr>
<td>Esophagus (mostly SCC)</td>
<td>50-82%</td>
</tr>
<tr>
<td>Gastric</td>
<td>34%</td>
</tr>
<tr>
<td>Liver</td>
<td>50%</td>
</tr>
<tr>
<td>Pancreas</td>
<td>83%</td>
</tr>
<tr>
<td>Colon</td>
<td>34%</td>
</tr>
<tr>
<td>Breast</td>
<td>41-66%</td>
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<tr>
<td>Prostate</td>
<td>40%</td>
</tr>
<tr>
<td>Renal</td>
<td>10%</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>10%</td>
</tr>
</tbody>
</table>

Liu L et al PLOS One 2016
Gonda et al Cell Dev 2010
Significant organ specific differences exist between CAFs.
Markers of CAFs

Other cells that express these markers:
- Pericytes
- Smooth muscle cells
- Neural cells
- Macrophages

Molecular markers:
- α-SMA
- FAPα
- NG2
- PDGFR-β
- Fibroblast–associated antigen
- Prolyl 4-hydroxylase

Terminology:
- Cancer-associated fibroblasts
- Myofibroblasts
- Fibroblasts

<table>
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<tr>
<th>Positive Marker</th>
<th>Negative Marker</th>
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<tbody>
<tr>
<td>α-SMA</td>
<td>Cytokeratin</td>
</tr>
<tr>
<td>Fibroblast activation protein</td>
<td>CD31</td>
</tr>
<tr>
<td>tenascin-C</td>
<td></td>
</tr>
<tr>
<td>periostin</td>
<td></td>
</tr>
<tr>
<td>Neuron glial antigen-2</td>
<td></td>
</tr>
<tr>
<td>Vimentin</td>
<td></td>
</tr>
<tr>
<td>Desmin</td>
<td></td>
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<tr>
<td>Platelet derived growth factor</td>
<td></td>
</tr>
<tr>
<td>receptor</td>
<td></td>
</tr>
<tr>
<td>Fibroblast specific protein-1</td>
<td></td>
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</table>
Stromal cells are present in the premalignant lesions

Origins of CAFs

• Activation or transformation of resident cells in the tumor
  – Activation of fibroblasts, smooth muscle cells, endothelial cells/pericytes
  – Epithelial mesenchymal Transformation

• Recruitment of bone marrow derived cells
Origins of CAFs

- Endothelial cells
- Epithelial cells
- Bone marrow derived hematopoietic stem cells
- Bone marrow derived mesenchymal stem cells
- Adipocyte

Transformations:
- endMT
- EMT

Resulting in:
- Resident fibroblasts
- Cancer associated fibroblasts
Activation of fibroblasts - tumor cell derived paracrine signals

TGF-β, PDGF, Shh, Wnt7a, Exosomes

IL-1β, LIF

α-SMA⁺

α-SMA⁻

α-SMA⁻

Tumor

Mezawa Cel Tiss Res 2016
Gonda Semin Cell Dev 2012
Activation of fibroblasts - tumor cell derived & autocrine signaling

MCF7 + fibroblasts -> CAF

CAF (+/- CXCR4 shRNA + MCF7)

Kojima Y PNAS 2010
Epithelial Mesenchymal Transformation (EMT)

- Observed in normal development/wound healing and cancer

Possibly reversible (E→M→E; EMT to MET)
Phenotypic similarities with CSCs

Significant epigenetic regulation of EMT-TFs

Kalluri Weinberg JCI 2009
Evidence for BM derived CAFs

Gastric Pre-Malignancy

WT
Gastri7s
Dysplasia

Pancreatic Cancer

Quante M et al Can Cell 2011

Direkze NC et al Can Res 2009
Significance of the origin of CAFs

• Heterogeneity in histologically (and to some extent phenotypically) identical cell populations
• Role in tumor stroma interactions and malignant transformations may be distinct based on cells of origin
• Importance for systemic targeted therapies in targeting BM derived cells (approximately 20-30%)
Stroma-Tumor Interaction

Synergy between stroma and epithelium in Pancreatic Cancer
CAFs but not normal tissue derived fibroblasts promote progression

CAFs independently contribute to epithelial malignant transformation

FSP.Cre

\[ FSP-1 \text{ Promoter} \]

\[ \text{EcoRI} \quad \text{HindIII} \quad \text{BamHI} \quad \text{DraIII} \quad \text{NotI} \]

\[ x \]

\[ \text{TGFBR2}^{\text{flox/flox}} \times 2 \]

\[ \text{Tgfrb2}^{\text{f/f}} \quad \text{Tgfrb2}^{\text{fspKO}} \]

Neil A. Bhowmick et al. Science 2004;303:848-851

Ki67
Why are CAFs independently tumorigenic?

Need to explain a stable phenotype
-Carcinogenic potential does not depend on presence of cancer cells

Possibilities:
-Genetic alterations
-Epigenetic changes
-Persistent autocrine stimulation
Stable stromal phenotype

• Genetic differences

Breast-Cancer Stromal Cells with TP53 Mutations and Nodal Metastases


• Epigenetic effect
Methylation profiling: cancer-associated myofibroblasts (CAFs) from gastric CA
Methylation profiling: cancer-associated myofibroblasts (CAFs) from gastric CA

Experimental samples

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<thead>
<tr>
<th>Case#</th>
<th>42</th>
<th>45</th>
<th>190</th>
<th>192</th>
<th>193</th>
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<tbody>
<tr>
<td>% methylated</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Jiang, Gonda et al Can Res 2009
Stromal DNA methylation in neoplastic progression

ASMA
Gastric Normal
Gastric Dysplasia
Gastric CA in situ

5meC

DNMT1
CAF
Epithelial
Panc1

Actin
Stromal DNA methylation in Pancreatic Cancer

Early PanIN

Late PanIN

vimentin/5mc/5Hmc

PanCA

PanCA

Relative 5mC intensity per nuclear area

Epithelium

CAF

Early PanIN

Late PanIN

S

E

DNMT1
DNA methylation in CAFs and epithelial cells in tumor progression

Normal

Dysplasia

Cancer

Stromal myofibroblast

Normal or malignant gastric epithelial cell

Methylated

Hypo-methylated
CAF and the tumor associated immune response

• Influence of biomechanical forces
  – Compression forces -> TNFα production -> CD4 activation
  – Tensile forces on fibroblasts -> activated MF phenotype and secretion of cytokines

• Paracrine signaling
  – Activated MFs/CAF express pro-inflammatory gene signature (CXCL1, CCL5, OPN, IL6, Il1B, CXCL12)
Recruitment of myeloid cells

- CAFs polarize towards M2>M1
  - Inhibition of CCL2 reverses this effect and restores recruitment of effector T cells

- Modification of ECM
  - HA synthetase conditional deletion in CAFs leads to depletion of TAMs

Hembruff Sl et al Neoplasia 2010
Kobayashi M et al Can Res 2010
Polarization of macrophage phenotypes

- **Older classification**
  - M1 (tumor suppressive)
  - M2 (tumor promoting)

- **Therapeutic goal**
  - Reprogram these cells to become immunostimulatory

Noy R et al Immunity 2016
Recruitment and activation of lymphocytes

• Cytokine release (CXCL9, CXCL10, CXCL12)
  • Balance of tumor suppressive (Th1, Th17) and promoting (NK, Th2) T cell recruitment

• ECM molecules
  • Tenascin- C effects T cell migration and activation

Ablation of activated CAFs (FAP +) results in a T cell dependent tumor suppression

FeigC et al PNAS 2013
CAFs influence (polarize) the tumor-immune response

Activation by biomechanical forces and paracrine signalling

Tumour-Suppressive

Tumour-Promoting

CTL  Th1  NK

Th2  Th17  Treg

M1

M2

Servais C and Erez N J Pathology 2013
Not all CAFs are created equal: Spatial distribution of inflammatory subsets

FAP positive CAFs produce most cytokines

FAP+ CAFs distant from epithelial cells are more likely to release cytokines then high SMA expressing cells adjacent to tumor

Yang X Can Res 2016
Ohlund D JEM 2017
Metabolic Alterations

• Differences in metabolic profile between CAFs and fibroblasts
  – Increased autophagy
  – Aerobic glycolysis -> release of “onco-metabolites: lactate ketones

• CAFs are capable of changing metabolic needs and pathways based on tumor needs
  – Opposite Warburg effect to epithelial cells

• CAF and epithelial cell metabolic pathways correlate with clinical outcome
CAFs fuel cancer cells – especially in hypoxic or nutrient-deplete conditions

- CAFs provide nutrients to tumor cells
- In nutrient-deplete environments the presence of stromal derived metabolites is more important then in normal conditions

CAFs remodel the ECM

- CAFs contribute to production of ECM
  - Structural component that may alter tissue stiffness and composition (HA)
  - Hedgehog signaling contributes to ECM production and Shh is not expressed in epithelial cells

- CAF may also regulate ECM degradation
  - MMPs lead to activation of VEGF, angiogenesis
  - uPA (urokinase Plasminogen activator) facilitates breakdown of ECM and invasion
Fluids, Solids and Gels in the microenvironment

• The gel-fluid phase (generated by accumulation of hyaluronan) leads to vascular collapse and is a possible cause of treatment resistance
CAFs beyond the primary tumor – role in metastasis

- CAFs establish a metastatic niche
  - Periostin (POSTN) modifies ECM and augments Wnt signaling
  - Tenascin C enhances Notch and Wnt signaling
- CAFs may induce EMT and facilitate invasion and metastatic dissemination
- At metastatic sites they may undergo MET and facilitate colonization
Summary of tumor promoting properties of CAFs

Ohlund et al. JEM 2014
Stromal cells – Hallmarks of Cancer

Hanahan and Coussens Cancer Cell 2012
Friend or Foe?
The Prognostic Value of CAFs in Human Cancer

- CAF density
- Expression profile of CAFs
- CAF/immune response
- Extracellular matrix composition and density
Association between CAF density and prognosis

Breast Ca
- Surokia P (2007)
  - 1.55 (0.62, 3.85)
  - 12.5
- Yamashita M (2012)
  - 1.16 (0.55, 2.44)
  - 18.6

Esophageal
- Ha SY (2014)
  - 2.04 (0.97, 4.29)
  - 18.8

Oral/H&N
- Fujii N (2012)
  - 1.68 (0.86, 3.30)
  - 23.0
- Ding L (2014)
  - 1.70 (0.69, 4.16)
  - 13.0

Pancreatic
- Sinn M (2014)
  - 2.24 (0.95, 5.26)
  - 14.2
- Overall
  - 1.68 (1.22, 2.32)
  - 100.0

ASMA positive cell densities

Meta-analyses

Association w/ disease free and progression free survival

Liu et al PLOS ONE 2016
CAF genes are associated with poor prognosis CRC

GSE33113, 14333, 39582

Genes associated with poor prognosis were significantly upregulated in LCM stroma

Calon E et al Nat Gen 2015
ECM composition and prognosis in Pancreatic Cancer

Whatcott et al Clin Can Res 2015

Median Survival
9.4 vs 24.3 mos

Median Survival
6.4 vs 14.6 mos

HA

Col I

Time (months)

Percent survival

Low HA

High HA

Low Col 1

High Col 1

Whatcott et al Clin Can Res 2015
PDAC associated immune response may be predictive of survival

- CD 86 (M1)
- CD 204 (M2)
- CD 66b+ (neutrophils)
- %Treg

Ino Y et al Br J Cancer 2013
Friend or Foe? Therapeutic Applications

- Rationale for CAF ablation
  - Association with poor prognosis
  - Synergy with epithelial cells
  - Barrier function for systemic chemotherapy
  - Possible synergy with tumor immune response
  - Impact of metastasis/metastatic niche

Neesse A et al Gut 2015
Friend or Foe?
Therapeutic Applications

• Approaches to targeting CAFs
  – Differentiation
  – Inhibition of CAF specific signaling
  – Cytokine release
  – Inhibition of ECM production
Proposed stromal targeted therapies

<table>
<thead>
<tr>
<th>Proposed Mechanism</th>
<th>Target</th>
<th>Drugs, drug classes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAF Differentiation</td>
<td><strong>DNMT1</strong></td>
<td><strong>5-aza-2’-deoxycytidine</strong></td>
</tr>
<tr>
<td></td>
<td>FAPα</td>
<td>Sibrotozumab</td>
</tr>
<tr>
<td>CAF-Epithelial Interaction</td>
<td>HGF/Met</td>
<td>NK4, anti-HGF mAbs</td>
</tr>
<tr>
<td></td>
<td>MMPs</td>
<td>Non-peptidic MMP inhibitors</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TIMPs</td>
</tr>
<tr>
<td></td>
<td>SDF1/CXCL2</td>
<td>AMD-3100</td>
</tr>
<tr>
<td></td>
<td><strong>Smo (Hedgehog pathway)</strong></td>
<td>IPI-926</td>
</tr>
<tr>
<td>CAF-ECM Interaction</td>
<td>MMPs</td>
<td>(as above)</td>
</tr>
<tr>
<td></td>
<td>Tenascin-C</td>
<td>Radioactive labeled antibody, siRNA</td>
</tr>
<tr>
<td></td>
<td>PAI-1/uPAR</td>
<td>Radioactive labeled PAI, Å6</td>
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<tr>
<td></td>
<td>FAPα</td>
<td>Sibrotozumab, small molecule inhibitors</td>
</tr>
<tr>
<td></td>
<td><strong>Hyaluronic Acid</strong></td>
<td>PEGPH20</td>
</tr>
<tr>
<td>CAF-Endothelial Interaction</td>
<td>PDGF-C</td>
<td>Antibodies used in synergy with anti-VEGF-A</td>
</tr>
<tr>
<td>CAF targeted anti-Inflammatory signaling</td>
<td>CD11b+ myeloid stromal cells (MDSCs)</td>
<td>CTL</td>
</tr>
<tr>
<td></td>
<td>COX-2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CXCL12</td>
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</table>
Targeting the stromal barrier and the ECM

- Inhibit ECM production (Shh pathway/ TGFB)
- Enzymatic degradation of hyaluronic acid
Enzymatic degradation of HA in model of pancreatic cancer

Proenzano PP et al Can Cell 2013
Encouraging Data with PEGPH20 plus AG*

- All patients received PEGPH20, Abraxane, gemcitabine and oral anticoagulant, rivaroxaban
- Primary endpoint: rate of TE events. No Grade 3/4 events reported
- Secondary endpoints:

<table>
<thead>
<tr>
<th>Response rate</th>
<th>57%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median PFS</td>
<td>8.4 months</td>
</tr>
<tr>
<td>Median OS</td>
<td>Not reached</td>
</tr>
<tr>
<td>Bleeding rate</td>
<td>7%</td>
</tr>
</tbody>
</table>

n=28

Kenneth Yu presentation ASCO GI 2018
Two tales of inhibition of Shh pathways in pancreatic cancer

IPI 926 inhibits SHH pathways in KPC model

Tumor Size: 5-10 mm

RX

Non tumor bearing (8 week old)

Olive KP et al Science 2009

Rhim A et al Can Cell 2012
Two tales of inhibition of Shh pathways in pancreatic cancer
Two tales of inhibition of Shh pathways in pancreatic cancer

- Inhibition of Shh pathway is a stromal mediated therapy
- Ablation of stroma results in regression of primary tumors
- (Early) ablation of stroma leads to a stroma poor, hypervascular, poorly differentiated cancer and increased mets
- Rationale for combination therapy targeting both CAFs and other components of TME
Targeting the CAF secreted factors

- Mitogens
- Chemokines
- Matricellular proteins
Targeting CAF-tumor cell interactions is essential for the efficacy of cytotoxic therapy

Increased accumulation of Gemcitabine is not sufficient to increase anti-tumor efficacy

Inhibition of Connective Tissue Growth Factor and Gemcitabine is sufficient to increase anti-tumor efficacy

Neesse et al PNAS 2013
Ablation of FAP enhances tumor immune response and uncovers anti-tumor effect

Genetic or pharmacologic ablation of FAP cells synergizes with TCIs

CXCL12 inhibition plus TCIs leads to regression and increased immune infiltration

Feig C et al PNAS 2013
Targeting CAFs

- Reversal of activated CAF phenotype
- Deactivation of CAFs
- Dedifferentiation of CAFs
  - Epigenetic strategies
  - Targeting (relatively specific) CAF expressing surface proteins
Hypomethylation therapy in pancreatic cancer

Pancreatic weight (grams)

% methylated HpaII sites in genomic DNA

Days

Percent survival

Shakya R et al Can Res 2013
Stroma ablating effect of Decitabine

**Graphs and Images:**
- **PBS control** vs **DAC escape**
- **p53**, **αSMA**, **p53/vimentin/TOPRO**
- **Graphs** showing %p53/HPF and % ASMA/HPF for PBS and DAC conditions.
- **Bar charts** showing %Ki67+ cells/hpf for Stromal and Epithelial conditions with PBS and DAC treatments.

**Statistical Significance:**
- **** for significant difference
- ***** for less significant difference
Decitabine modifies tumor immune response in addition (or as a result of) stromal ablation

Significant IFN-γ response

Polarization of tumor associated immune response

Significant benefit of Decitabine plus TCI therapy
Conclusions

• Tumor stroma plays a role in all stages of carcinogenesis
• Both clinical outcome/prognosis and treatment success impacted by stromal changes
• Genetically more stable, epigenetically altered and epigenetically modifiable targets
• Heterogenous population – both within a tumor and in between different cancers
Conclusions II

• Delicate balance of tumor suppressing and promoting properties
  – CAFs may initially support then restrain cancer
  – Enhancing tumor immune response through stroma ablation may be a particularly effective approach

• CAF targeted therapies are probably necessary to be an adjunct to effective chemotherapy but unlikely to be effective independently