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
- Gastritis
- Dysplasia

Pancreatic Cancer

- WT

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

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

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

\[
\times
\]

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

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

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

• 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/CAFs express pro-inflammatory gene signature (CXCL1, CCL5, OPN, IL6, Il1B, CXCL12)
Recruitment of myeloid cells

• Cytokine release
  – CCL2 inhibition in (Breast)CAFs leads to tumor suppression via decrease of TAM
  – CAFs polarize towards a tumor promoting macrophage phenotype

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

Hembruff SI et al Neoplasia 2012
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

CAFs in influence (polarize) the tumor-immune response

CAFs have been established as a key component of the crosstalk between malignant tumour cells and their microenvironment. Central to their role in facilitating tumour growth, invasion, and metastasis is their ability to orchestrate tumour-promoting inflammation. Due to the vast heterogeneity of fibroblasts, as well as their different origins, much of the accumulating knowledge on the functional roles and activation pathways of fibroblasts may be tumour type- and tumour stage-specific. Nevertheless, clinical oncology is progressing increasingly towards a new era of integrative cancer therapy, based on personalized diagnostics that takes into account the individual complexities of tumours, including cells, pathways, and molecular mediators in the tumour microenvironment. As a result, cancer therapeutics is moving progressively towards combinatorial approaches that act synergistically by targeting intrinsic pathways in neoplastic cells, as well as extrinsic tumour-enabling pathways in the tumour microenvironment. Future studies will decipher in detail the molecular pathways underlying the activation/recruitment of fibroblasts to become pro-inflammatory CAFs supporting early carcinogenic progress, as well as CAF functions that enable the formation of metastases, and will hopefully result in innovative therapeutic strategies allowing the co-targeting of immune cells and CAFs to maximize treatment efficacy and prevent evasive resistance.

Acknowledgments

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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 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
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
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

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

ASMA
Gastric Normal
Gastric Dysplasia
Gastric CA in situ

5meC

DNMT1

DNMT1
Actin