Stromal microenvironment in tumor development & progression

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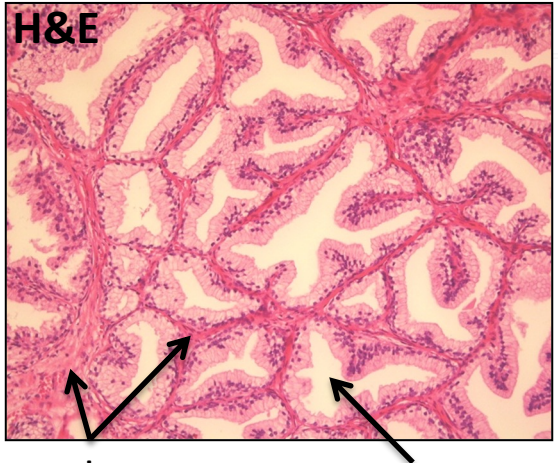
Overview

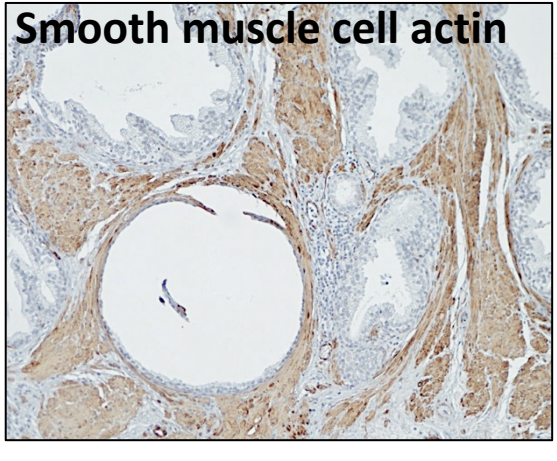
1. Background
   - Stromal tissue: definition, composition, function
   - Molecular/cellular changes in tumor microenvironment
   - Cancer-associated fibroblasts as major tumor-modulating entities in tumor microenvironment
2. Paper presentation
3. Take home message

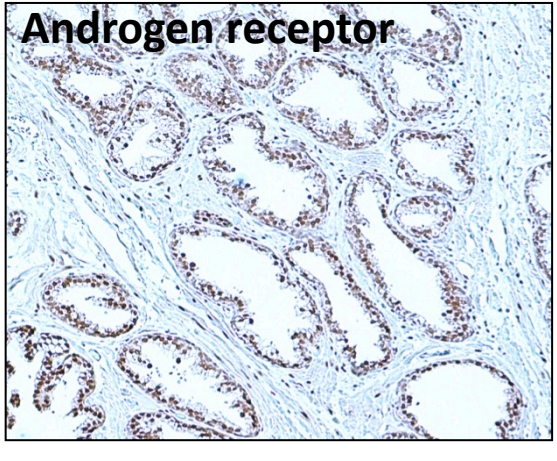
Stroma

Definition:

- the supportive framework or matrix of a cell or organ
- comes from Greek meaning **bed covering or mattress**







70% of prostate = stroma

Stroma: composition & origin

- stroma derives from embyronic mesenchyme

cellular component:
nerves
blood vessels
immune cells
fibroblasts
smooth muscle cells (SMCs)

non-cellular component:
connective tissue
extracellular matrix (ECM)

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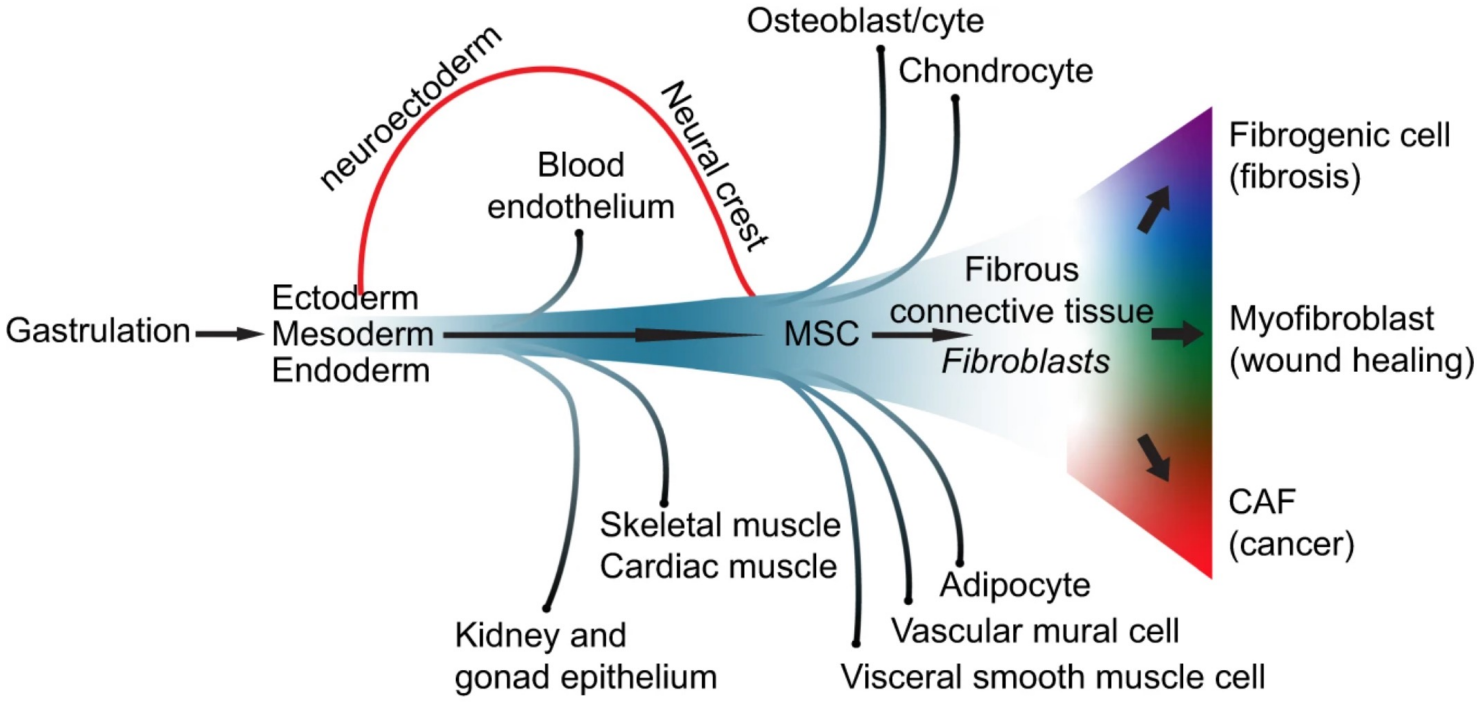
Stromal cell origins

From: Identification, discrimination and heterogeneity of fibroblasts



Other cell types originating from the mesoderm are also depicted. MSC are shown as a transitory cell type that yield fibroblasts. In fibrosis, wound healing and cancer, fibroblasts likely progress further to become fibrogenic cells, myofibroblasts and CAFs, respectively.

Lendahl et al *Nat Comm*: **13**, Article number: 3409 (2022)

Stroma: function

1. provides structural support/rigidity to organ and tissue (connective tissue)

2. key regulator of tissue homeostasis & organ development:
   - reciprocal interactions between epithelium and stroma via paracrine-acting signaling molecules (growth factors and cytokines)
   - signaling cascades regulate epithelial and stromal cell proliferation, differentiation, ECM production, angiogenesis (i.e. key processes that contribute to cancer)

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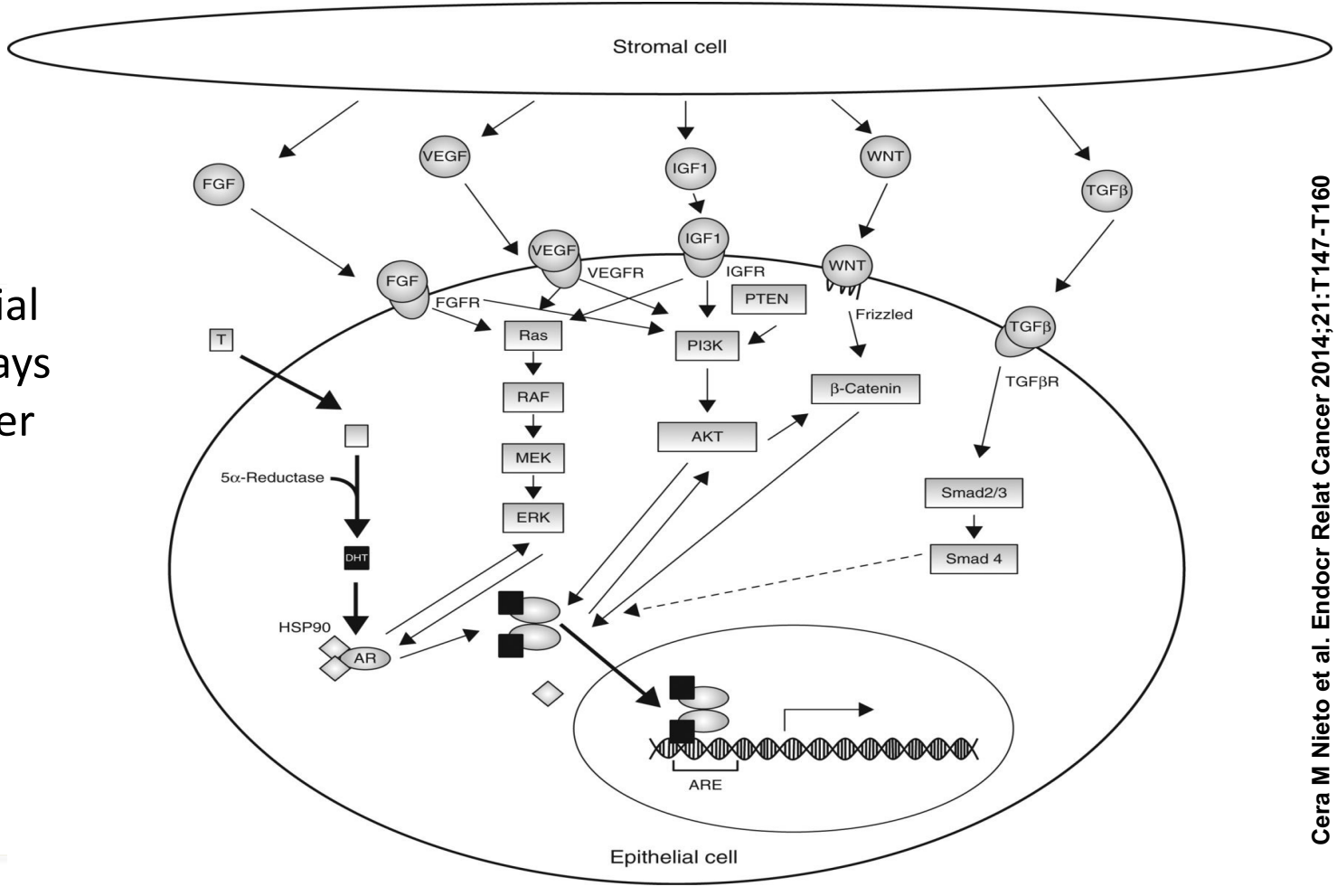
Stromal – epithelial interactions

EXAMPLE:
AR-regulated stromal–epithelial signaling pathways in prostate cancer



Cera M Nieto et al. Endocr Relat Cancer 2014;21:T147-T160

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Tumor stroma: historical perspective

1863: Rudolph Virchow observed leukocytes in stroma of neoplastic tissue. He hypothesized that malignancy originated at sites of chronic inflammation

1889: Paget noted that some tumor cells (the "seed") grow preferentially in the **microenvironment** of selected organs (the "soil") and that metastases only result when the appropriate seed is implanted in its suitable soil

1924: Max Borst wrote „with regards to the question of whether the epithelium or the connective tissue has the leading role in carcinogenesis, we think that asking "*either/or*" is bad."

Tumor-promoting microenvironment

- Tumor stroma enhances tumorigenicity
- Inoculated cancer cells embedded in tumor stroma are 10 – 100 fold more tumorigenic than stroma-free suspensions of cancer cells

Table 1. *Tumorigenicity of Cancer Cells Inoculated into Normal Mice as Cell Suspensions or Fragments of Solid Tumors*

Tumor	Inoculum*	Tumor cells	Tumor outgrowth‡
		$\times 10^6$	
1591-PRO	Suspension	50	0/7§
	Suspension	10	1/8
	Fragments	15	11/15
	Fragments	3	10/12
	Fragments	1.5	8/12§
6134A-PRO	Suspension	50	0/5‖
	Suspension	10	0/16
	Fragments	15	9/11
	Fragments	3	8/12
	Fragments	1.5	7/12‖

Singh et al. 1992 J Exp Med 175: 139

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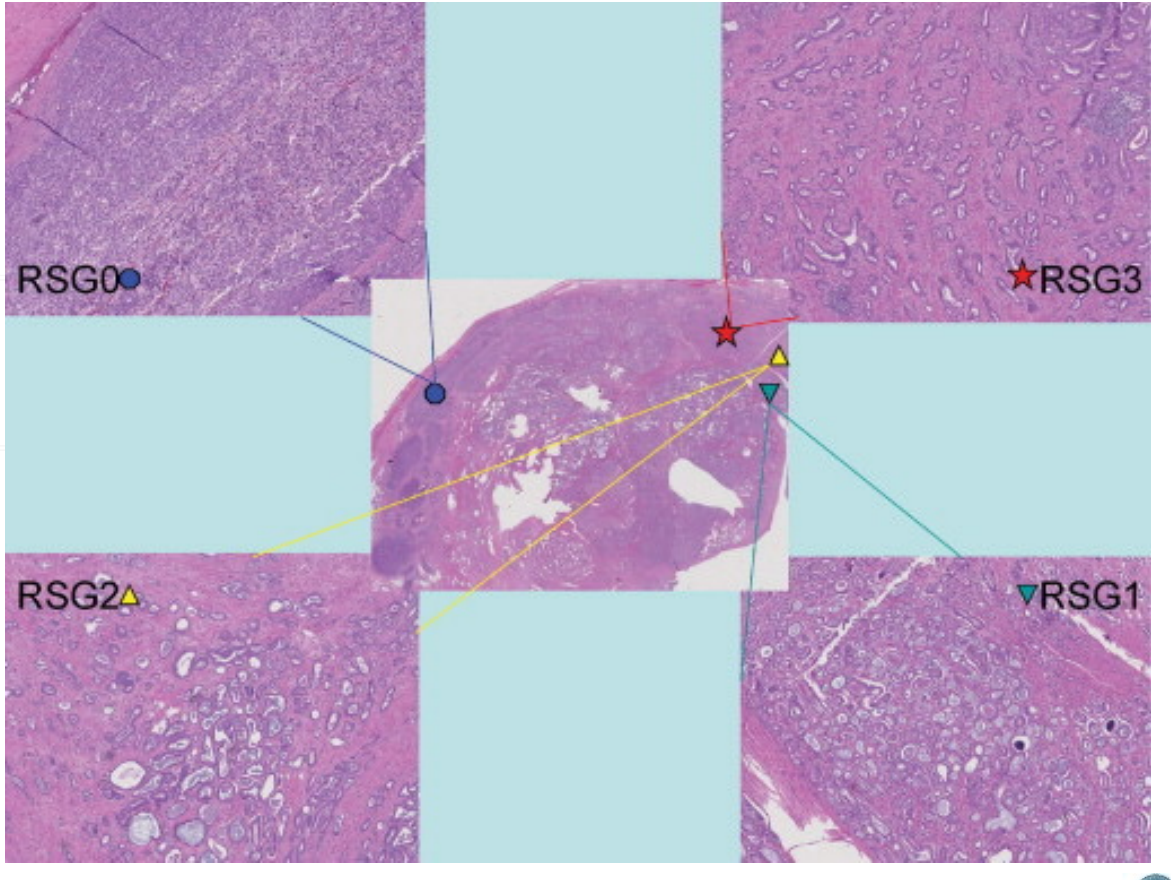
Reactive stroma grading

4 reactive stromal grades (RSG) based on
% area of reactive stroma in the tumor

RSG	% RS : tumor
0	≤5%
1	6% – 15%
2	16% – 50%
3	≥1:1 ratio



Ayala et al. 2011 Am J Pathol 178: 79-87.

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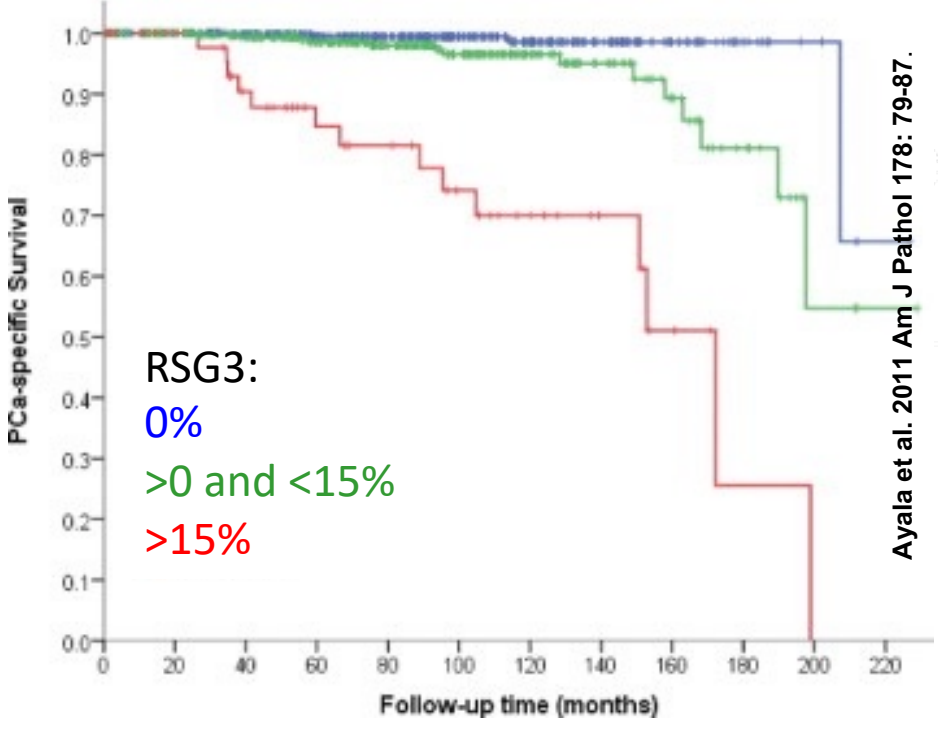
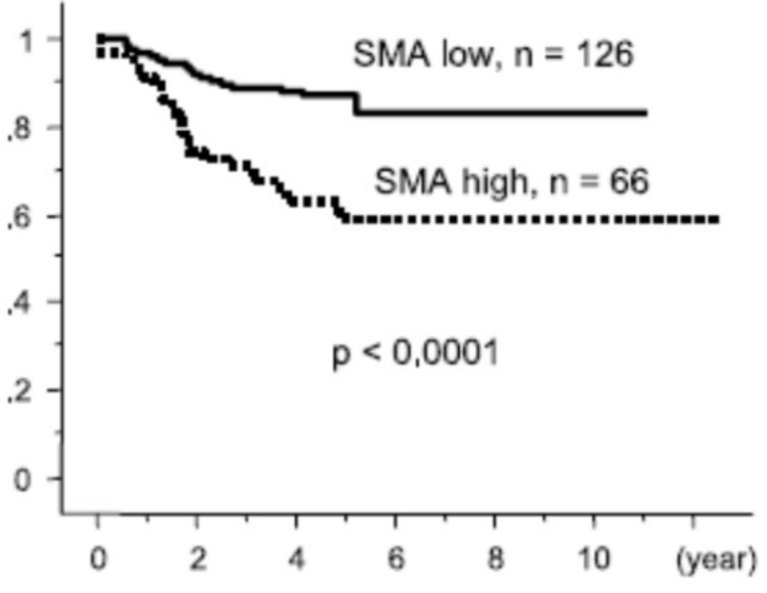
Reactive stroma: clinical relevance

prostate cancer-specific mortality



Ayala et al. 2011 Am J Pathol 178: 79-87.

overall survival - colorectal cancer



(Tsujino et al., Clin. Cancer Res. 2007)

- stroma-enriched tumors are associated with poor prognosis

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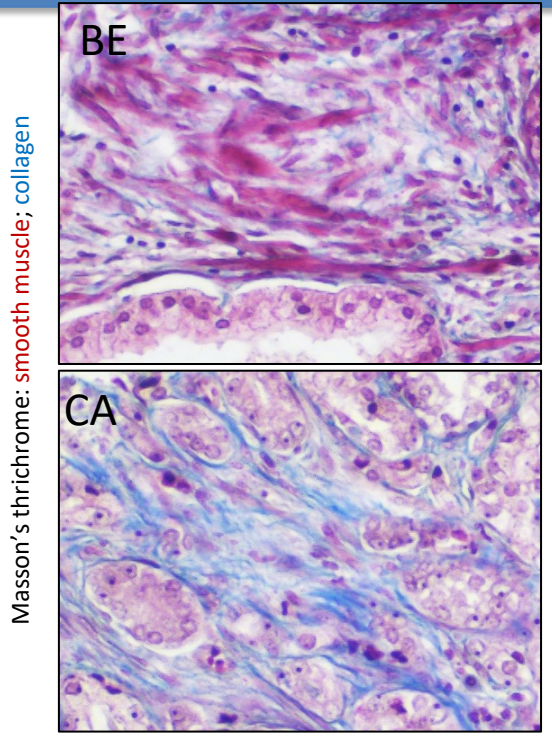
Tumor microenvironment

- reactive stroma exhibits histo-morphological hallmarks:

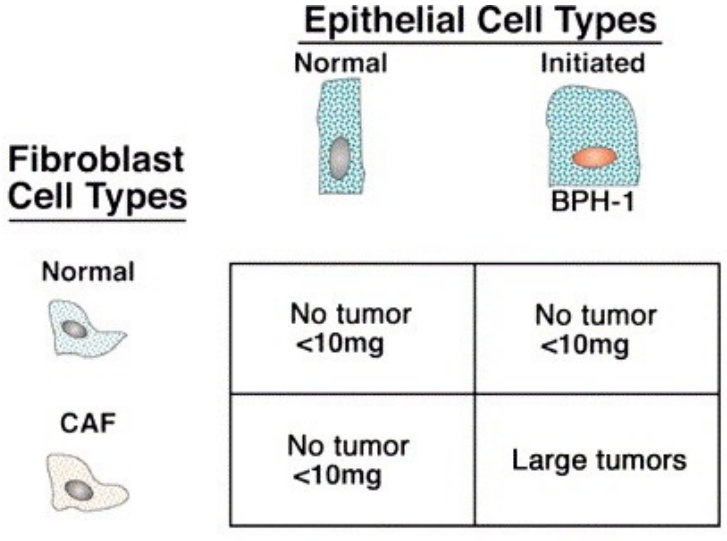
each required for tumor development

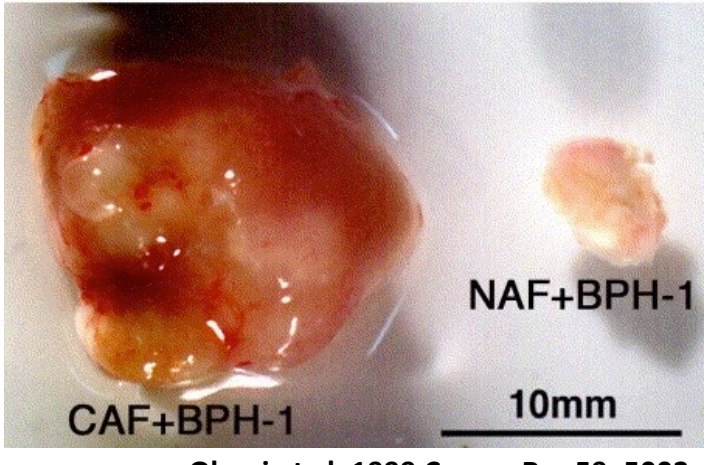
  - presence of carcinoma-associated fibroblasts (CAF, activated phenotype)
  - increased deposition of altered ECM
  - increased capillary density (aberrant structure/ leaky vessels)
  - immune cell infiltration



- changes apparent in pre-neoplastic lesions (early event in tumorigenesis)
- tumor-associated stroma changes (co-evolves) during tumor progression

Carcinoma Associated Fibroblasts



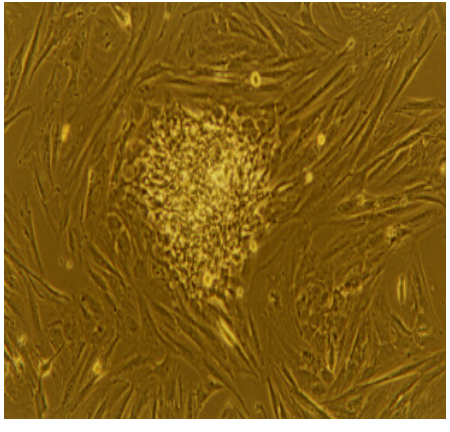


Olumi et al. 1999 Cancer Res 59: 5002

- Tumor-promoting capacity of stroma predominantly mediated by CAF

CAF

- persistently activated fibroblast-like cells in stroma adjacent to the tumor and at invasive front (spindle-like morphology)



- CAF phenotype proven by ability to promote tumorigenesis of initiated but non-tumorigenic epithelial cells

- Isolated from tumor biopsies via
  - (i) outgrowth from tissue slices in media containing serum
  - (ii) tissue digestion with collagenase and differential centrifugation/FACS

CAF: molecular hallmarks

- exhibit widespread DNA hypomethylation
- no single molecular marker to define CAF
- common markers include:

fibroblast activation protein (FAP)	platelet derived growth factor receptors
alpha smooth muscle actin (SMA)	Tenascin C
fibroblast specific protein (FSP1)	podoplanin
CD90/Thy1	↓ caveolin-1

- not all CAF express these markers (different CAF subtypes) and these markers are not necessarily CAF-specific
- CAF subtypes also exhibit functional differences

CAF heterogeneity

- „CAF“ represents a heterogeneous mix of functionally-distinct cell types/cell states
- Cytokine/expression profiles characterize different CAF subtypes
- CAF can promote or inhibit tumor progression:
  - *podoplanin$^+$ CAF = poor prognosis in lung adenocarcinoma, squamous cell carcinoma and breast cancer*
  - *CD90 (THY1)$^+$ CAF = tumor-promoting in prostate cancer*
  - *FAP$^+$ CAF = associated with poor outcome in colon cancer*
  - *FAP$^+$ CAF depletion = increased tumor progression in mouse PDAC model*
  - *inhibiting Shh signaling = promoted tumor aggressiveness in mouse PDAC model*

➢ Different subtypes of CAF exist, which are functionally/molecularly distinct - heterogeneity may reflect different activation stimuli (e.g. IL6 vs. TGFβ) and/or CAF cellular origin (i.e. local resident fibroblast vs. infiltrated BM-derived cell)

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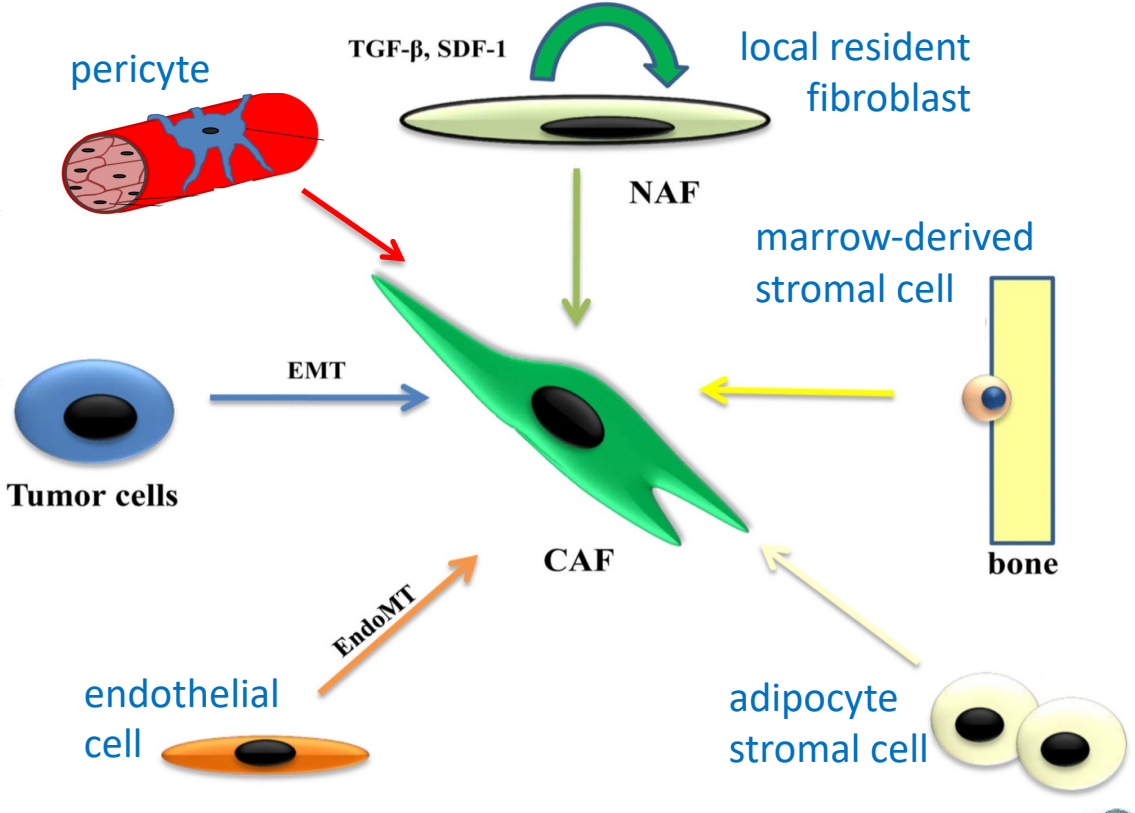
CAF cellular origins

CAF derive from:

1. activation of local resident fibroblasts

2. chemokine-attracted infiltrating cells



Mao et al. 2013 Cancer Metastasis Rev 32: 303

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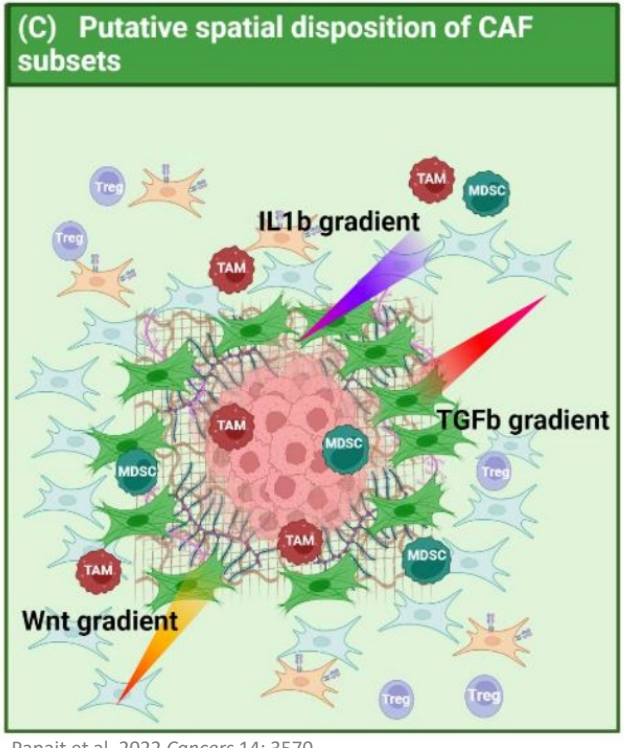
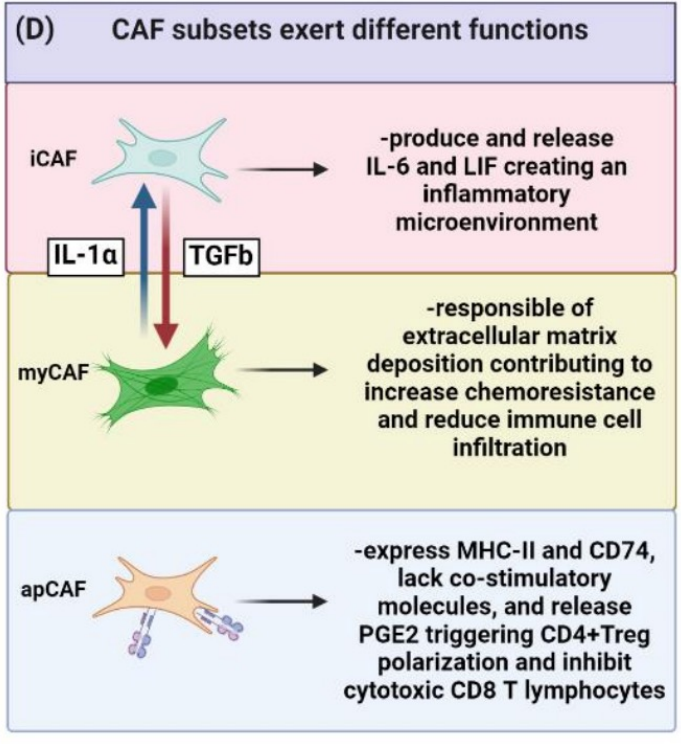
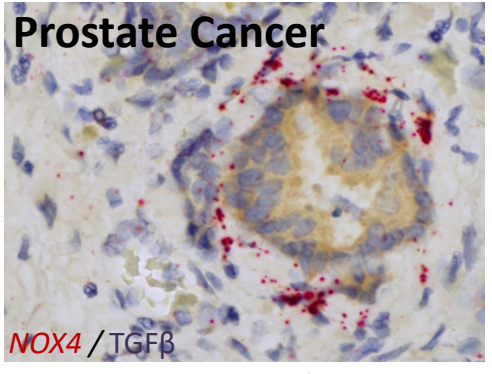
Prevailing CAF substates





Papait et al. 2022 *Cancers* 14: 3570



Sampson et al. 2018. Int J Cancer

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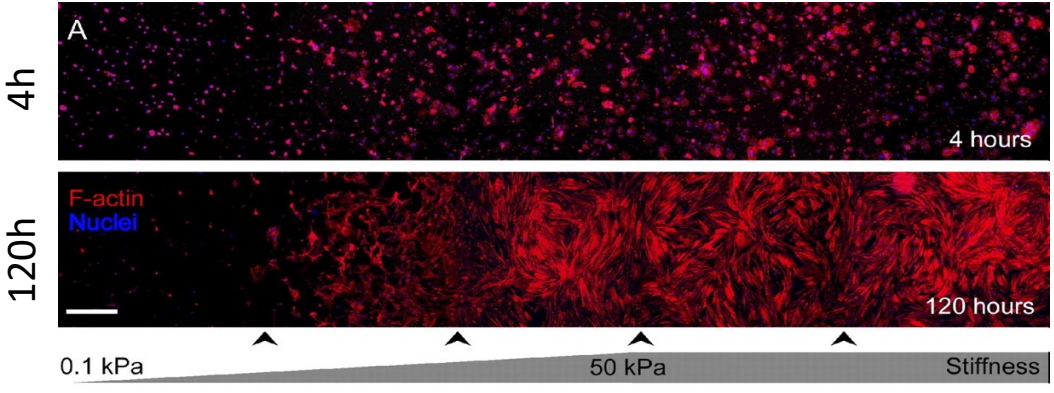
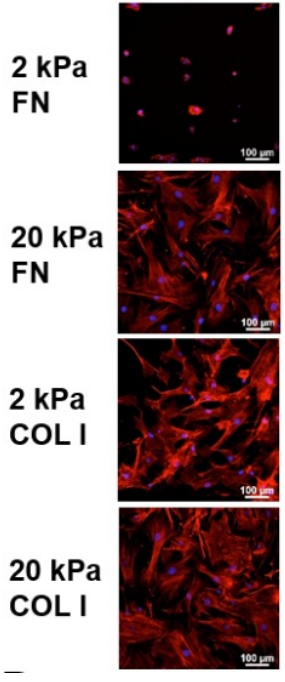
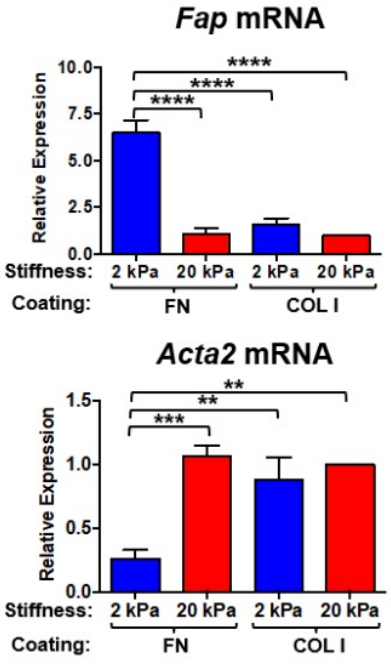
Matrix stiffness & ECM substrate regulate CAF polarization







Molecular basis: changes in mechanotransduction → integrin switching, focal adhesion formation & intracellular signaling

https://repository.upenn.edu/cgi/viewcontent.cgi?article=3959&context=edissertations
J Cell Biol. 2010;190(4):693-706. doi:10.1083/jcb.201004082

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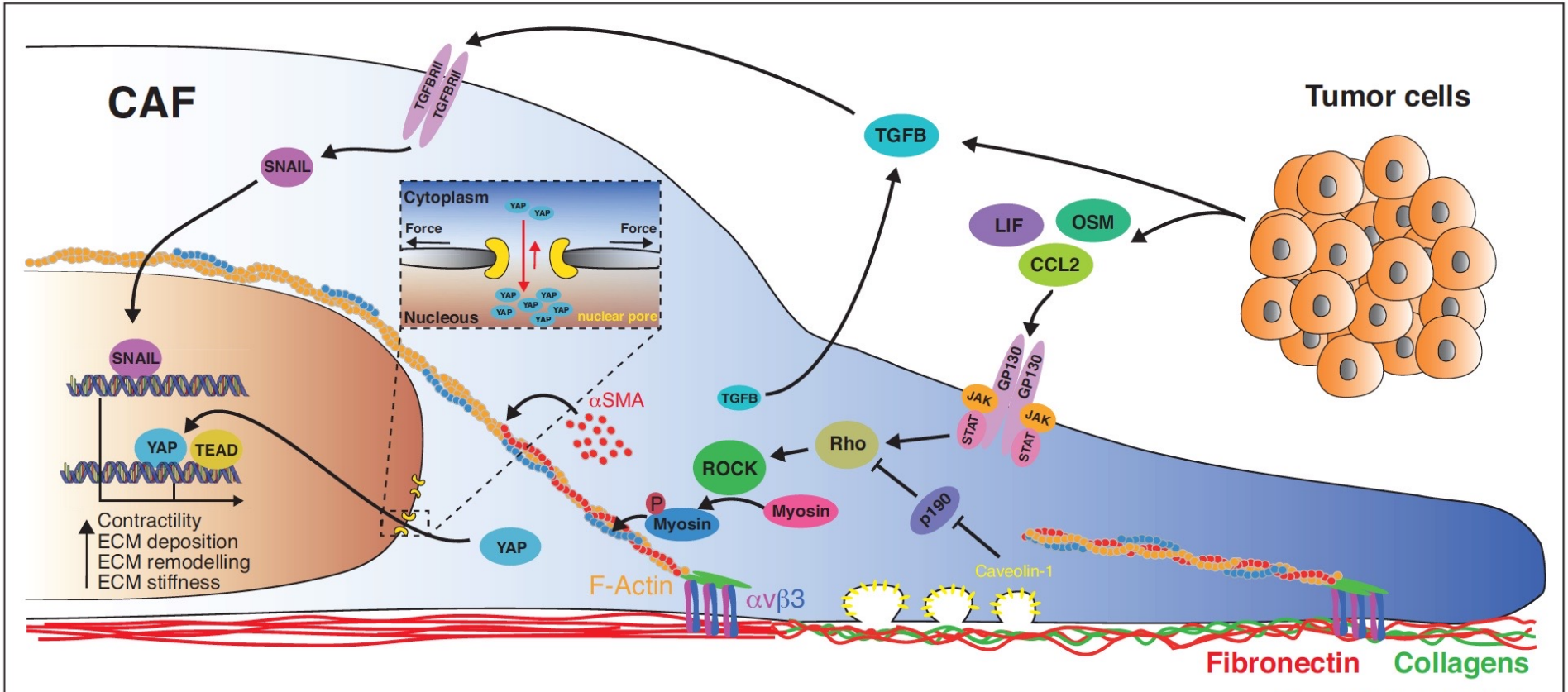
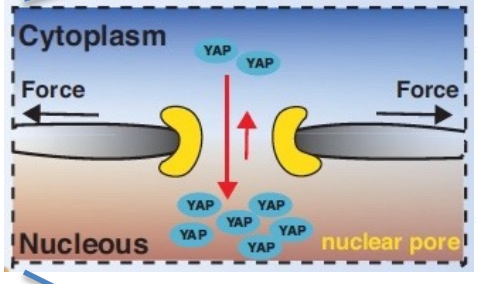
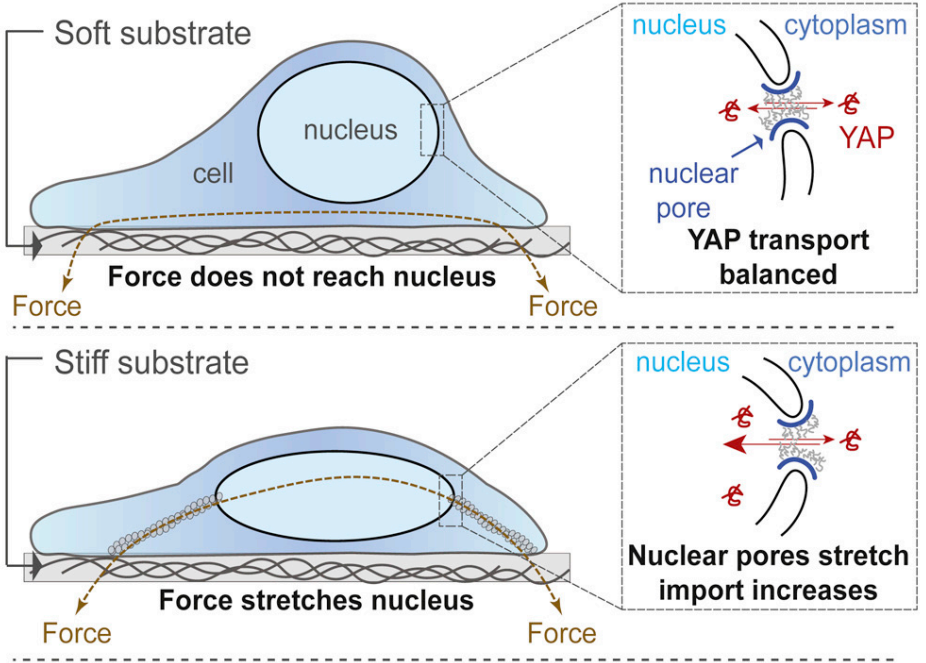
ECM remodeling and CAF contractility



Barbazan and Vignjevic 2019 Current Opinion in Cell Biol 56: 71-79

Mechanical activation of YAP





Elosegui-Artola et al. 2017 Cell 171:1397

Downstream CAF actions

Direct mechanisms

- secrete paracrine-acting soluble factors e.g. IL-6
- direct cell-cell contacts with tumor cells

Indirect mechanisms

- ECM deposition / remodeling
- angiogenesis
- modulate immune response
- metabolic reprogramming

Therapy resistance

- reduced chemotherapeutic efficacy
- endocrine/target resistance

➢ CAF target for novel approach of stromal-targeted anti-cancer therapies?

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CAF-induced ECM remodeling/deposition

- CAF-derived fibronectin (ECM component) attract tumor cell engagement

Erdogan et al. (2017) J Cell Biol 216: 3799

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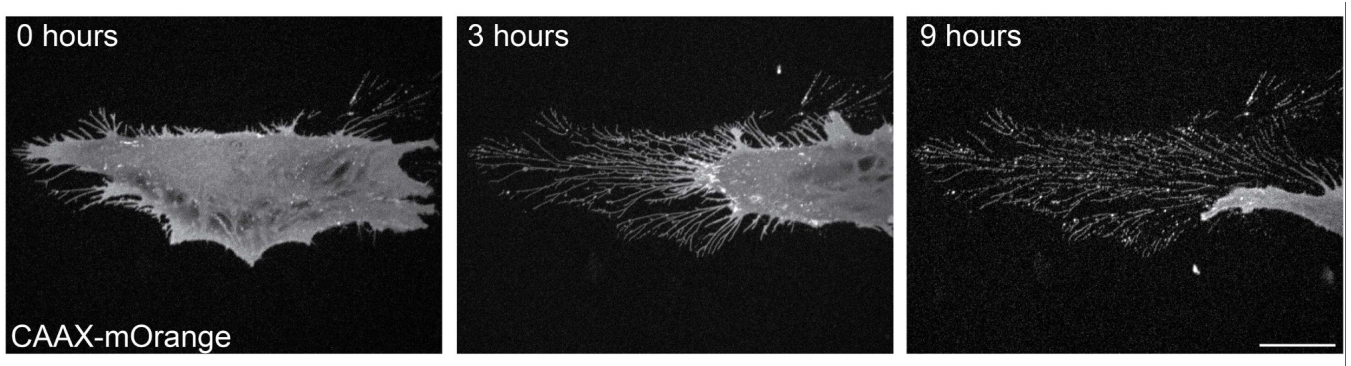
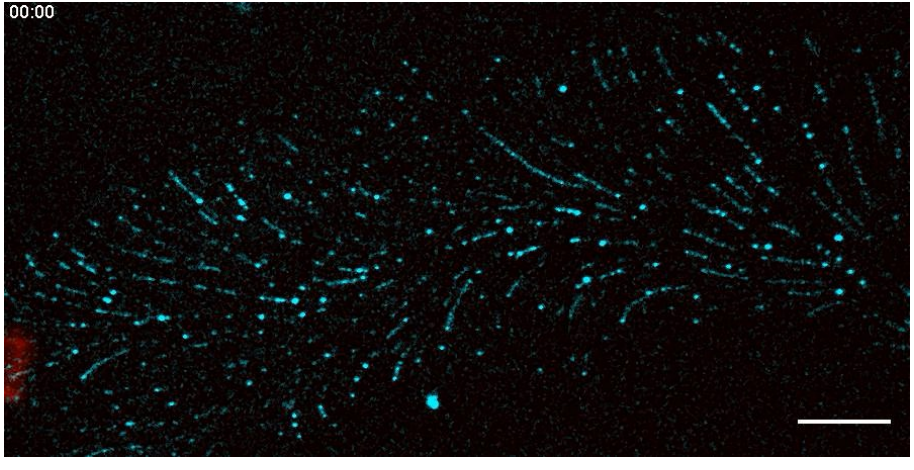
CAF-induced ECM remodeling/deposition

- CAF produce ECM tracks along which tumor cells migrate





Baschieri et al. (2023) Sci Adv 9: eade2120

➔ CAF-led tumor cell migration

- ECM stiffness/enhanced integrin-mediated cell adhesion promotes invadopodia formation (protusions that direct tumor cell invasion)

- stiffened ECM promotes TGFβ-induced EMT thereby enhancing metastasis

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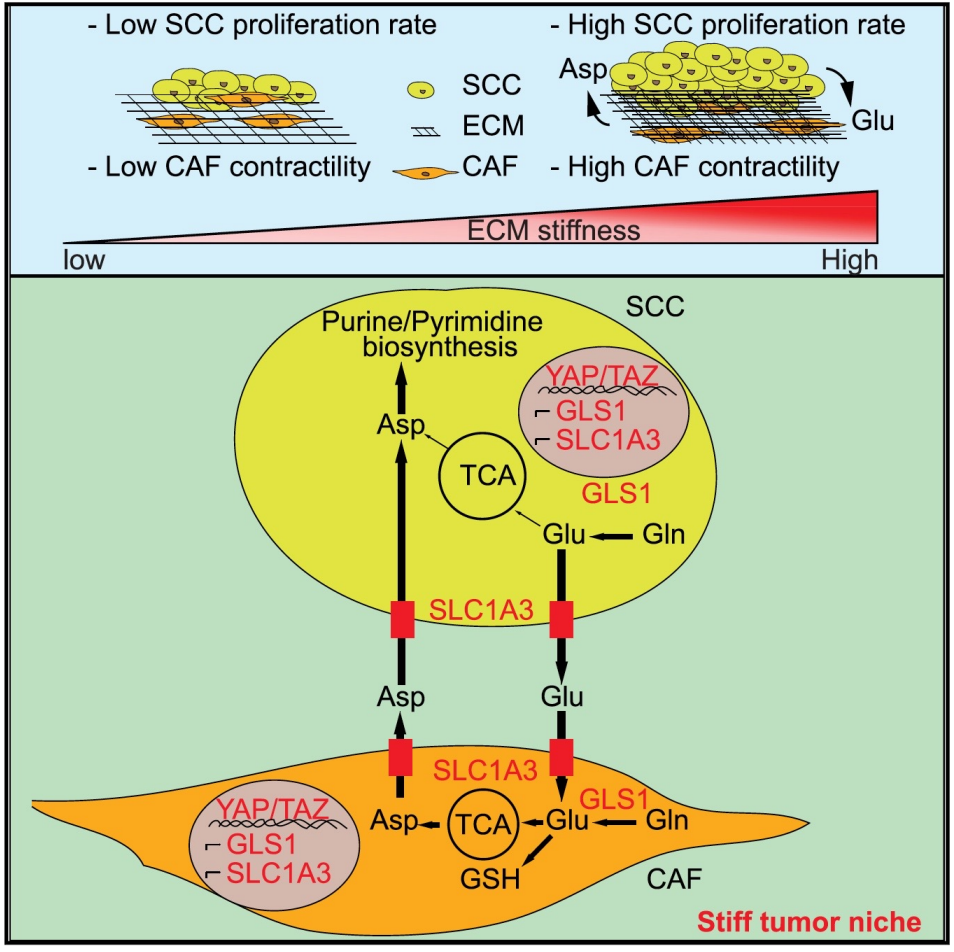
CAF-driven ECM remodeling influences metabolic crosstalk

- Tumor niche stiffening induces a metabolic switch in cancer cells and CAF
- ECM stiffening linked to metabolic re-wiring via a YAP/TAZ-dependent glutamate/aspartate crosstalk
- Mechanotransduction coordinates amino acid exchange within the tumor niche
- Aspartate/glutamate crosstalk sustains tumor cells and stromal fibroblast activation
- Amino acid exchange supports tumor growth and metastasis *in vivo*



Cancer Metab (2018) **29**: 1-17

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Paper

nature communications

Article

https://doi.org/10.1038/s41467-024-47068-z

Deciphering the spatial landscape and plasticity of immunosuppressive fibroblasts in breast cancer

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Hugo Croizer[1,2,7], Rana Mhaidly[1,2,7], Yann Kieffer[1,2,7], Geraldine Gentric[1,2], Lounes Djerroudi[1,2,3], Renaud Leclere[3], Floriane Pelon[1,2], Catherine Robley[1,2], Mylene Bohec[4,5], Arnaud Meng[1,2], Didier Meseure[3], Emanuela Romano[6], Sylvain Baulande[4,5], Agathe Peltier[1,2], Anne Vincent-Salomon[3] & Fatima Mechta-Grigoriou[1,2]

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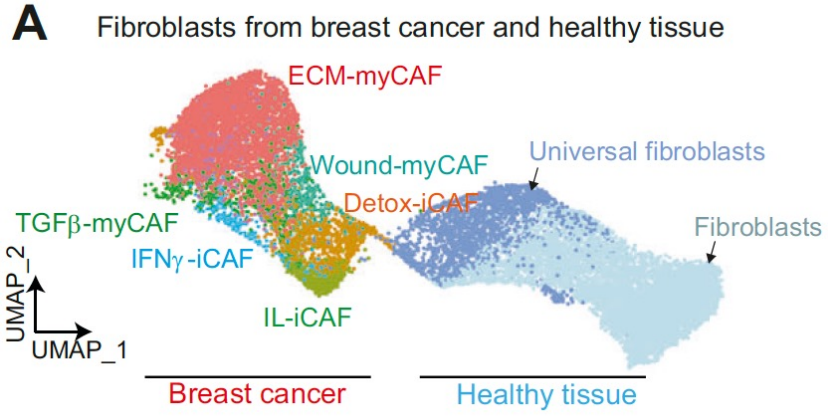
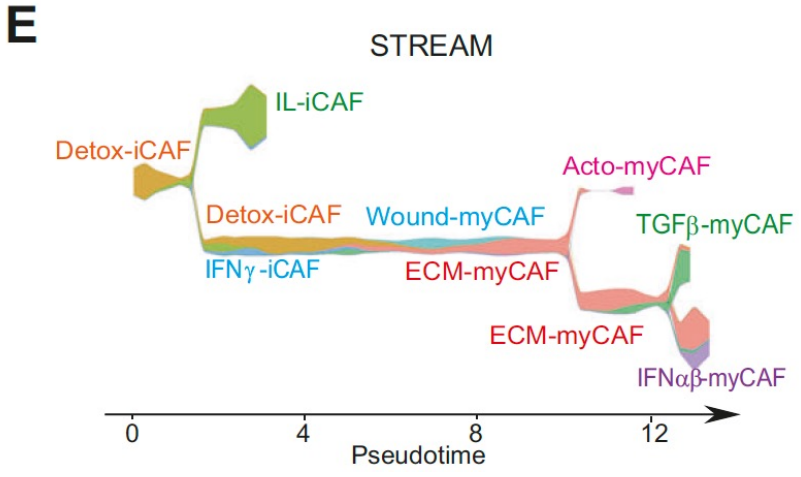
Background / Aim

Breast cancer heterogeneous disease:

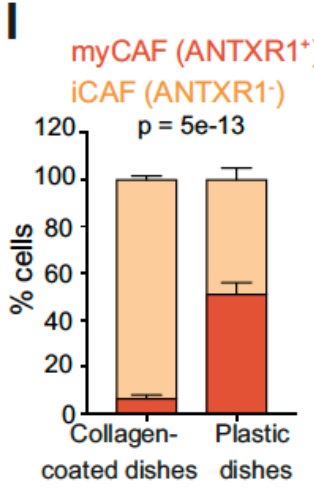
- luminal, HER2, triple-negative subtypes
- no biomarkers to predict progression from ductal carcinoma in situ (DCIS) to invasive breast cancer (IBC)
- myofibroblastic CAF (myCAF) associated with BC progression
- FAP^+ CAF population comprised of 8 cellular subclusters (3x iCAF, 5x myCAF)
- immune cells not randomly distributed in tumors but organized into niches (can predict response to immunotherapy)

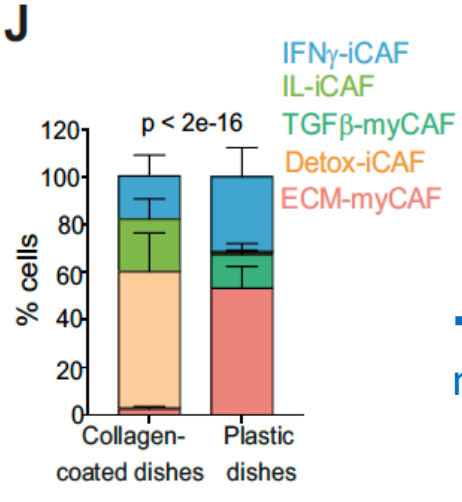
$\Rightarrow$ Uncover plasticity and spatial organization of CAF with other cell types



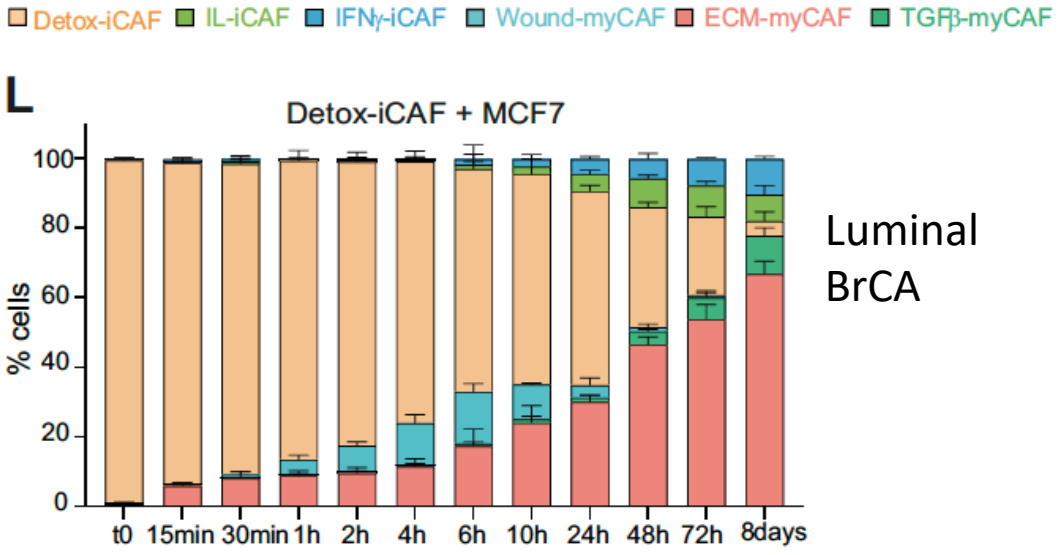


detox cluster = progenitor CAF

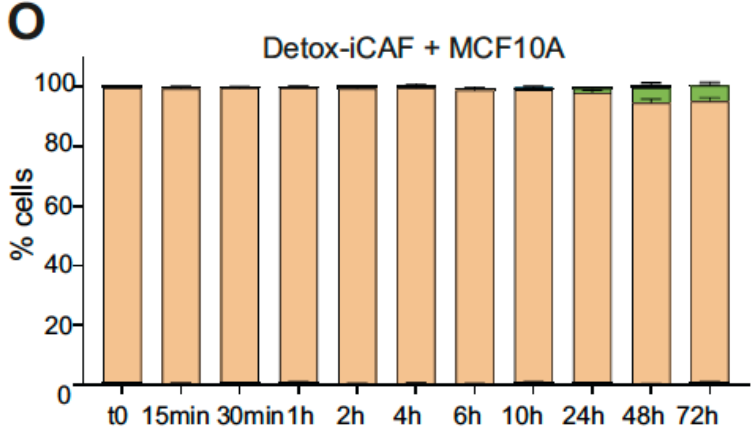




➔ stiff matrix promotes myCAF formation



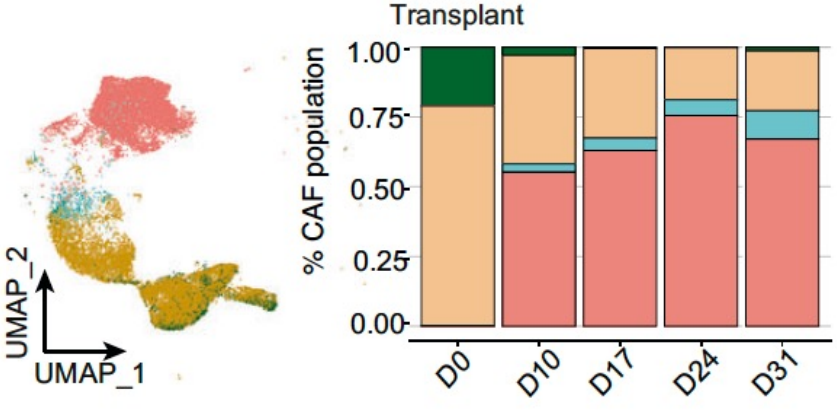
Luminal BrCA



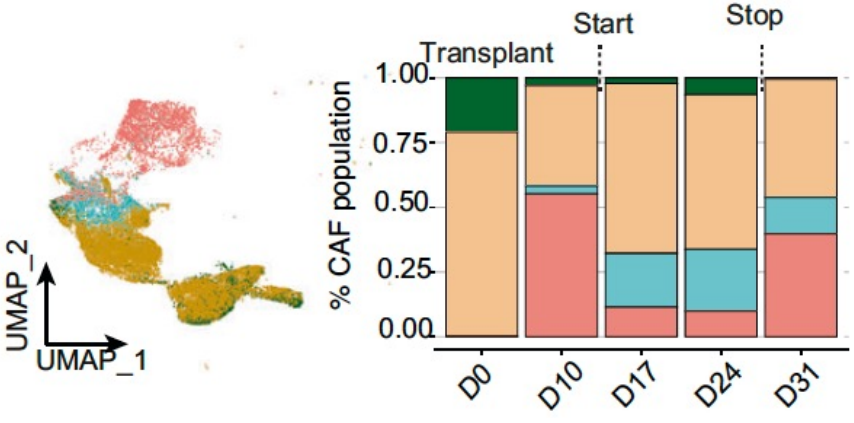
non-tumorigenic breast epithelial cell line

- Tumor cells induce detox-iCAF to phenotypically switch to myCAF substate(s)

PDAC mouse model



Lrrc15+ depletion in PDAC mouse model



xenotransplanted PDAC tumor cells

→ myCAF accumulate over time

(LRRC15 is myCAF marker)

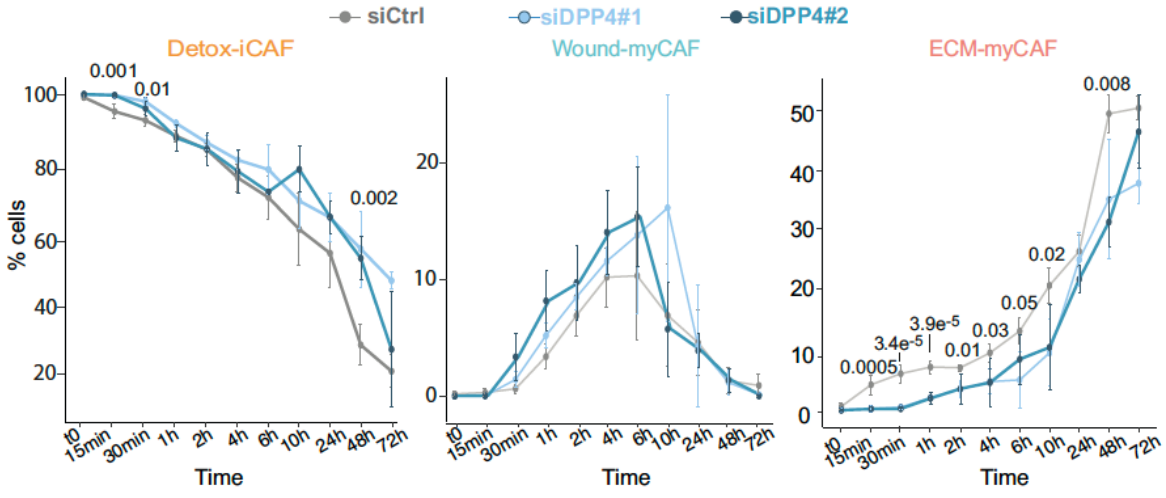
xenotransplantated PDAC tumor cells

→ myCAF accumulate over time

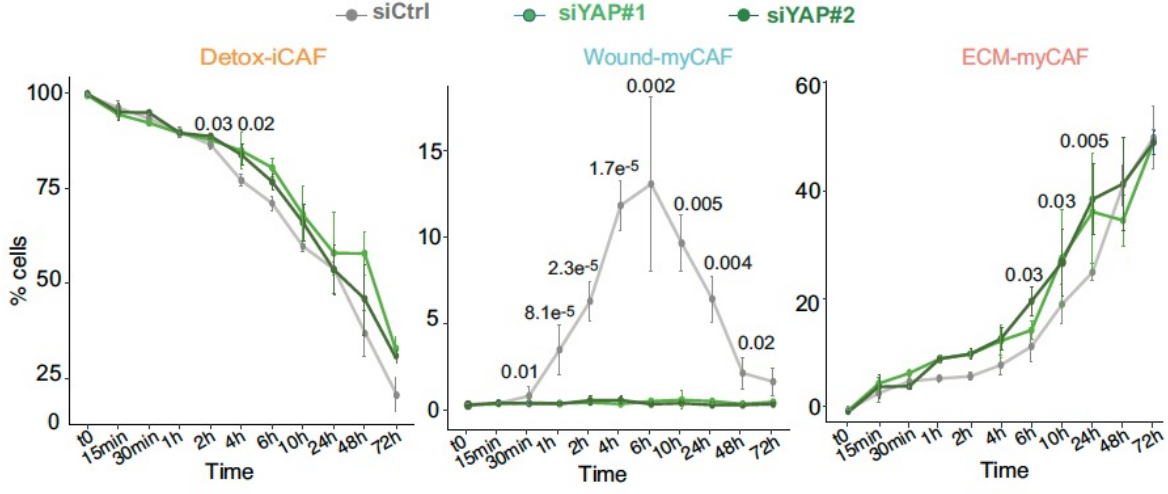
→ depleted myCAF (diptheria toxin)

→ upon treatment cessation, myCAF return

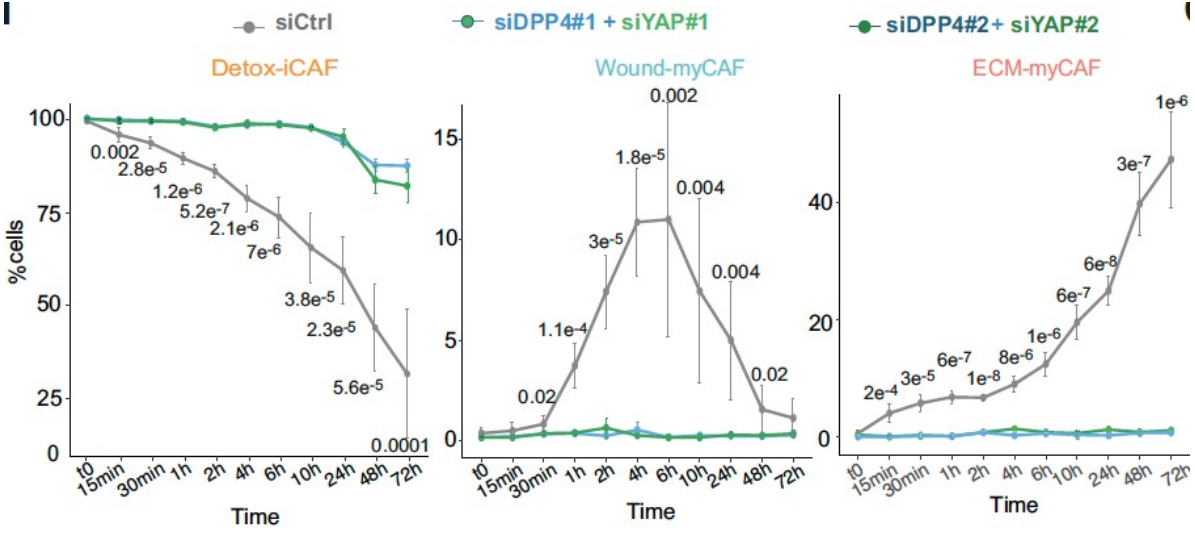
CAF substates following co-culture with BrCA cells:



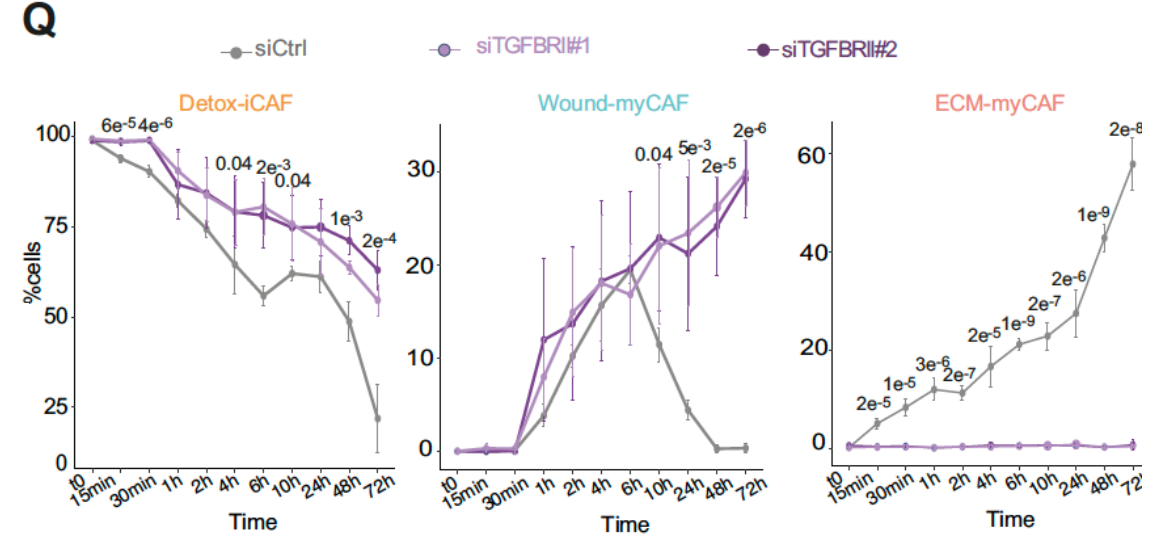
Delayed kinetics → DPP4 required for initial transition to ECM-myCAF state



YAP is required for intermediate wound-myCAF state (ECM-myCAF state can arise independently of YAP)

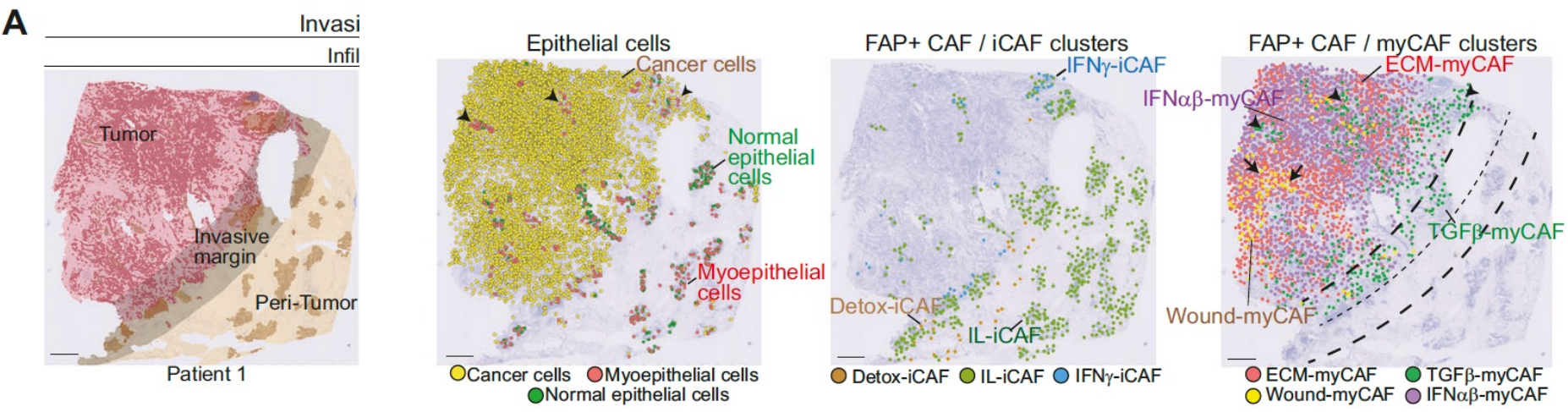


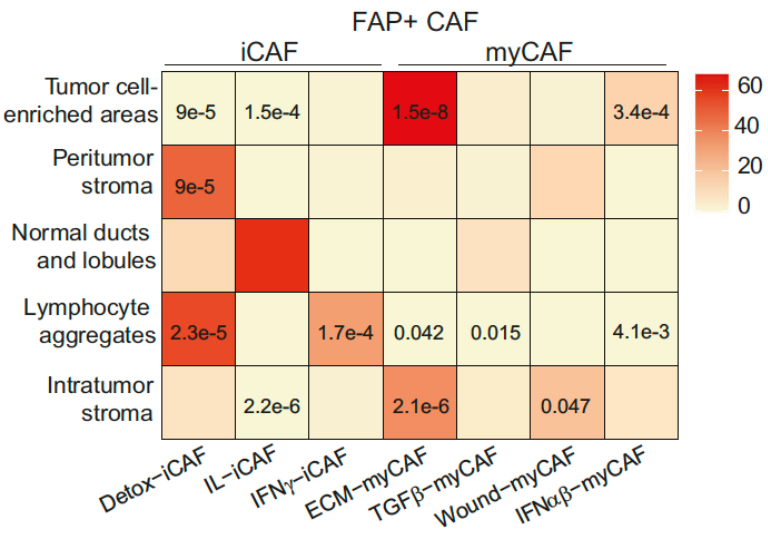
DPP4 and YAP are required for iCAF transition to intermediate & late-myCAF states



Critical role of TGFb signaling in wound-myCAF formation

➔ Interaction between DPP4/YAP/TGFb signaling pathways drive CAF plasticity

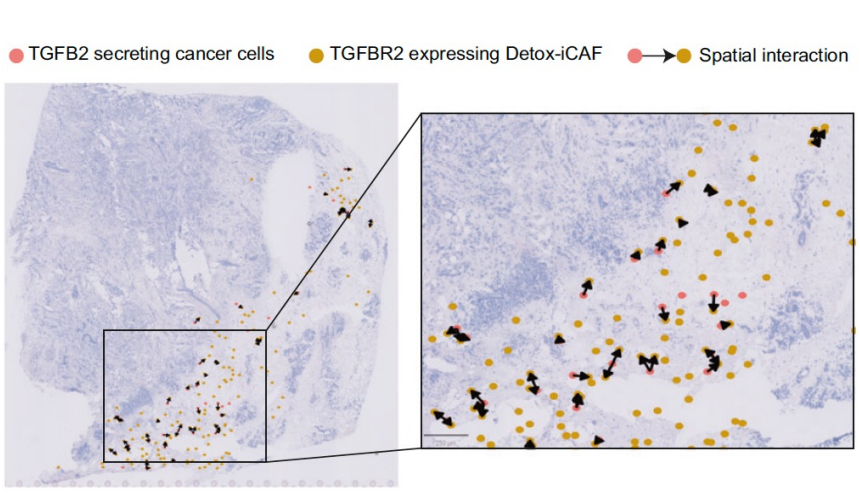




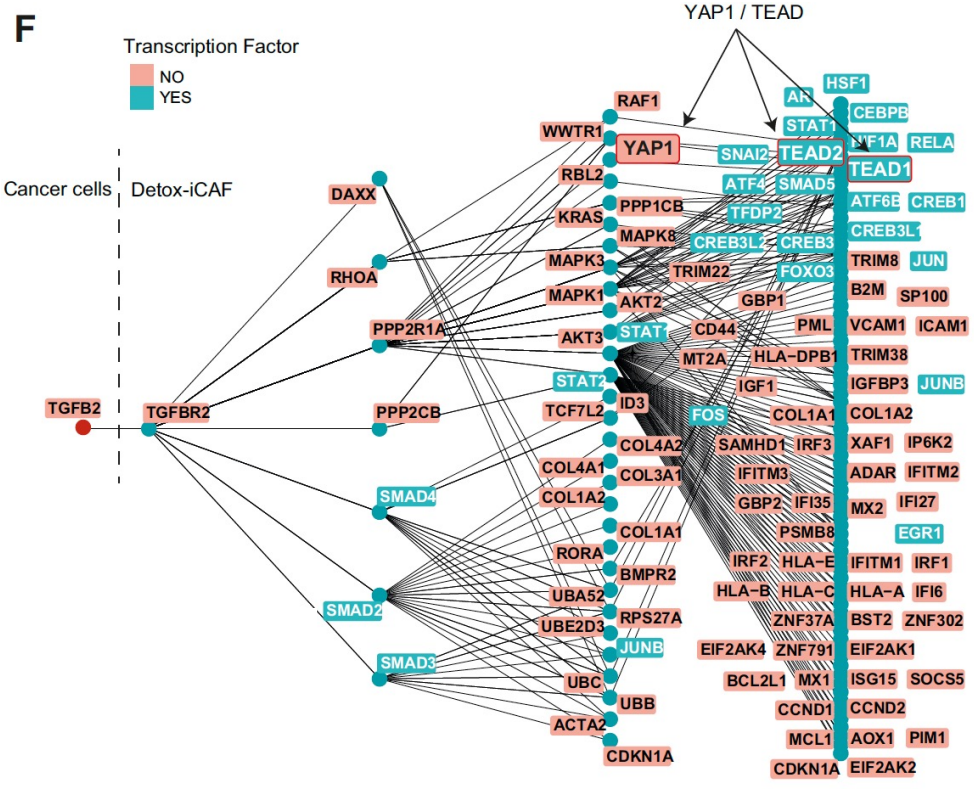
iCAF substates more distal to tumor, enriched around blood vessels

myCAF substates within tumor bed in close contact with tumor cells

➔ CAF occupy distinct spatial niches within the TME

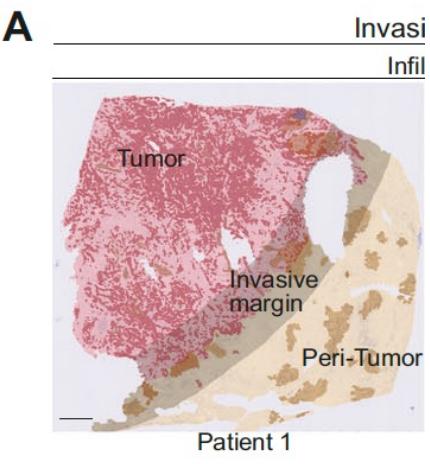


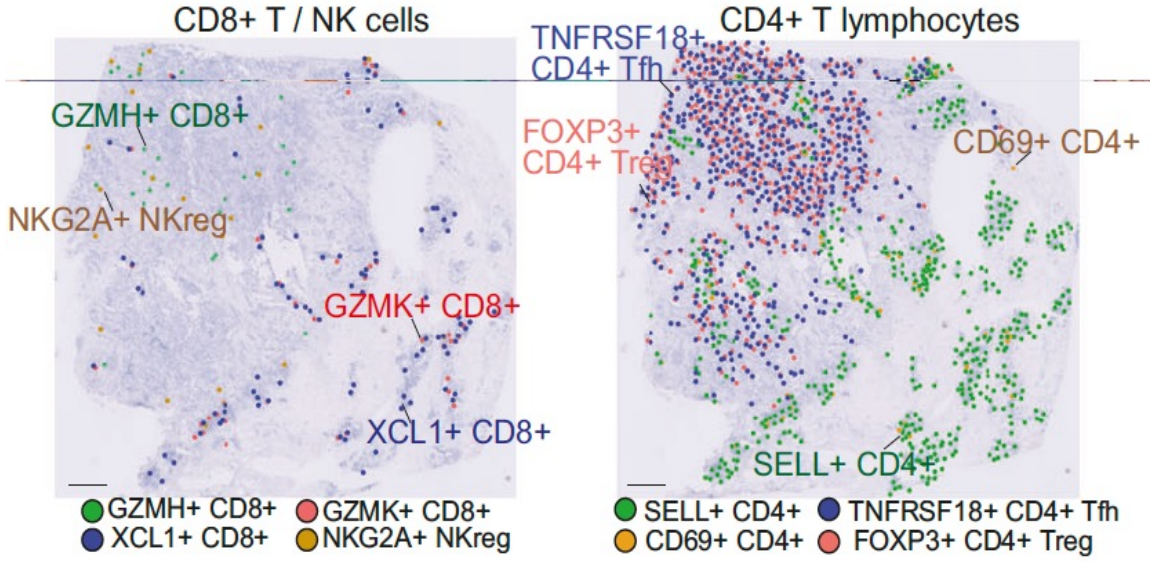
TGFB2 secreting tumor cells at the invasive front are in close proximity to TGFBR2⁺ detox-iCAF



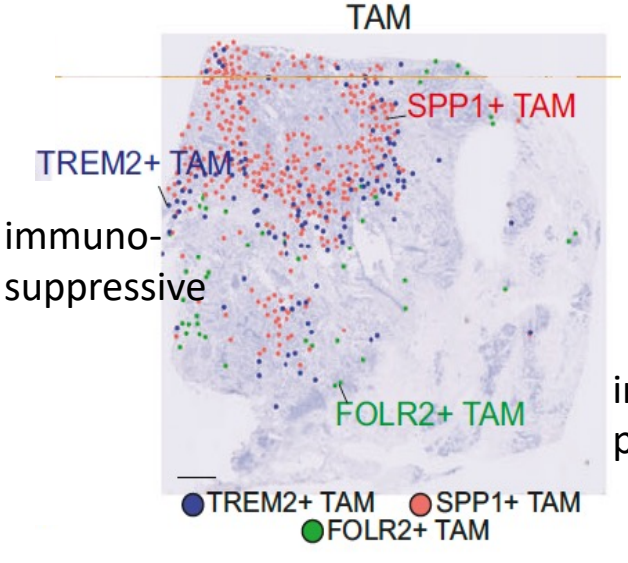
downstream pathways activated by TGFB2-TGFBR2 interaction

- Tumor cells at the invasive front initiate TGFb and YAP signaling in neighboring stromal cells driving myCAF transition





lymphocyte aggregates in tumor bed
(site of myCAF accumulation)



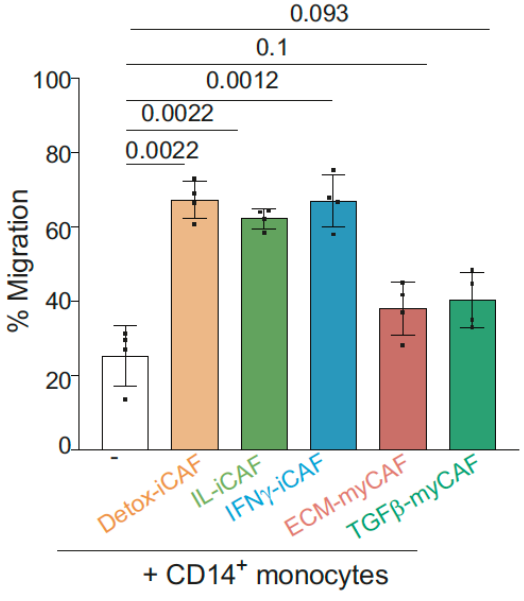
immuno-suppressive

immuno-protective

TAM within tumor bed

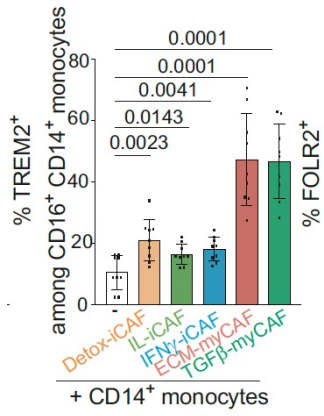
➔ immune cells occupy distinct spatial niches within the TME

Co-cultured different CAF substates with CD14⁺ monocytes/CD4⁺ T cells:

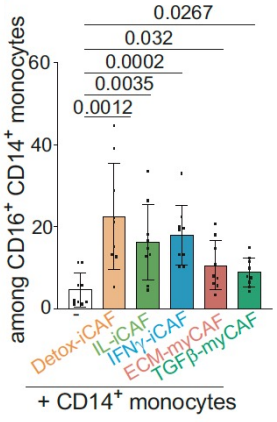


➢ iCAF attract monocytes

TREM2⁺



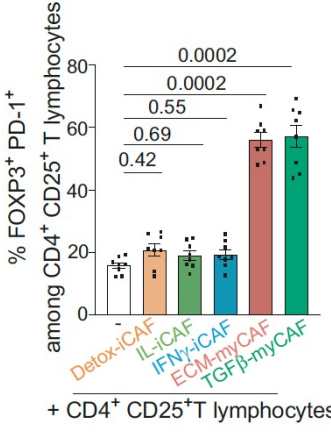
FOLR2⁺



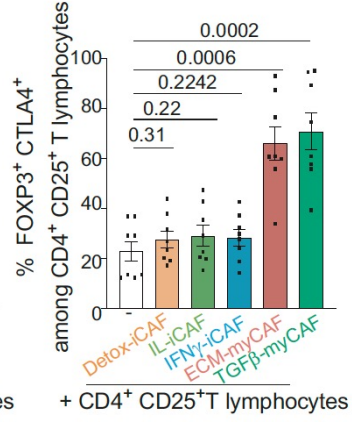
➢ iCAF promote immuno-protective niche

➢ myCAF promote immuno-suppressive TAM

PD1⁺



CTL⁺



➢ myCAF promote immuno-suppressive TREG formation

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TAKE HOME MESSAGES

- Stromal microenvironment is dynamic and integral part of solid tumors that plays key role in tumor development/progression, immune suppression, therapy resistance and clinical outcome
- Tumor stroma not just an innocent bystander but an active driver of tumor progression
- Altered paracrine signaling by CAFs is major effector mechanism underlying tumor-promoting actions of the tumor-associated stroma (effects on tumor cells, ECM remodeling, angiogenesis and immune suppression)
- Cellular components of the TME occupy distinct niches → evolving concept of cellular micro-neighborhoods
- Restoring paracrine signaling networks between stromal components/eradicating specific CAF subpopulations may represent anti-cancer therapeutic strategy