Cellular Signaling and Cancer Plasticity Group - CPG

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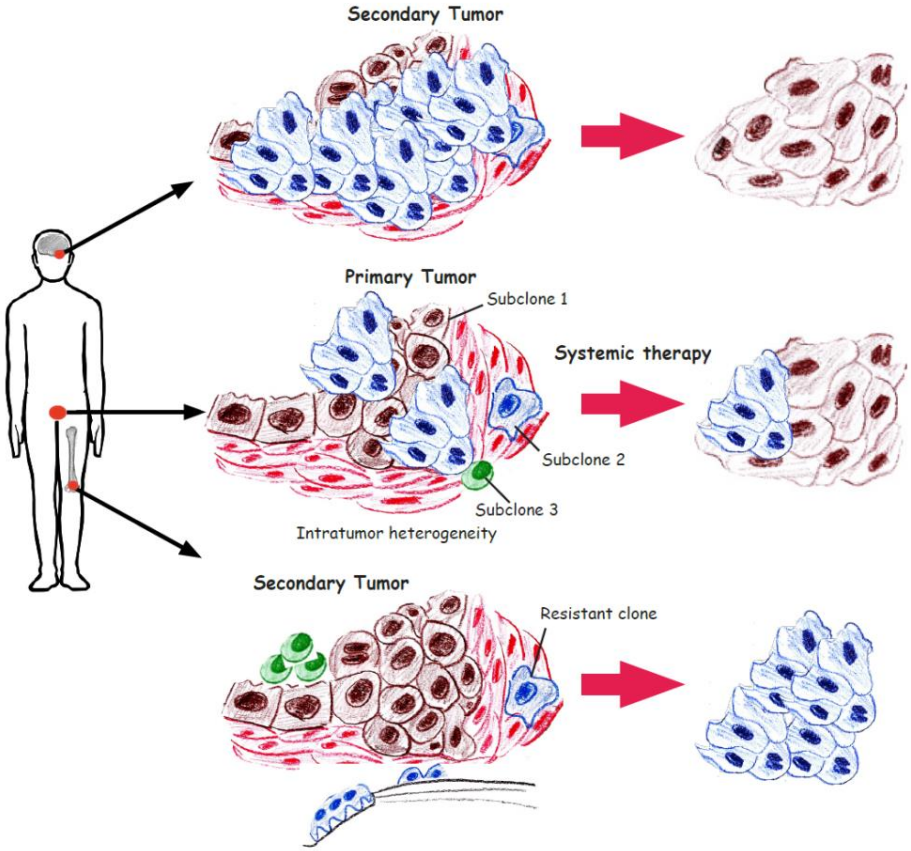
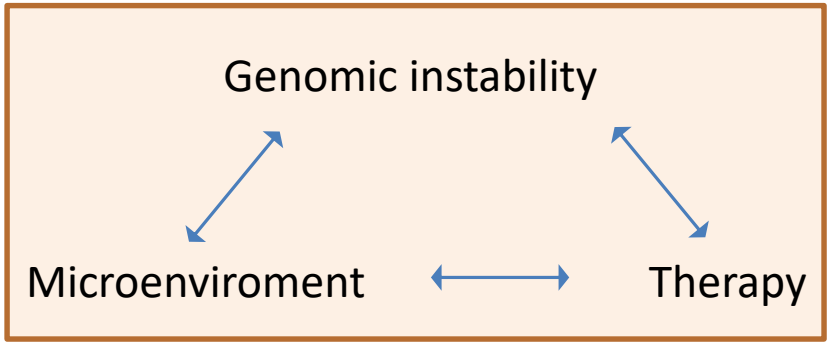
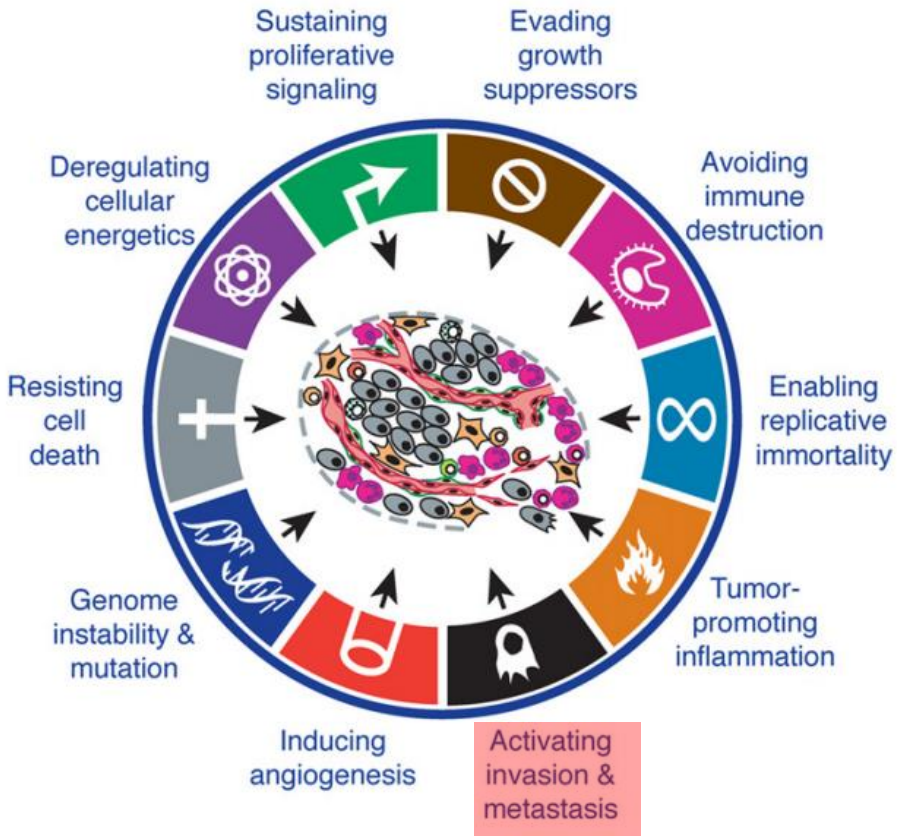
Plasticity of cancer cells

- Cancer is heterogeneous and not single cell disease.
- Complex and dynamic, NOT static “ecosystem”.
- Diversity inside tumors is clinical problem limiting the efficacy of targeted therapies and compromising treatment outcomes.



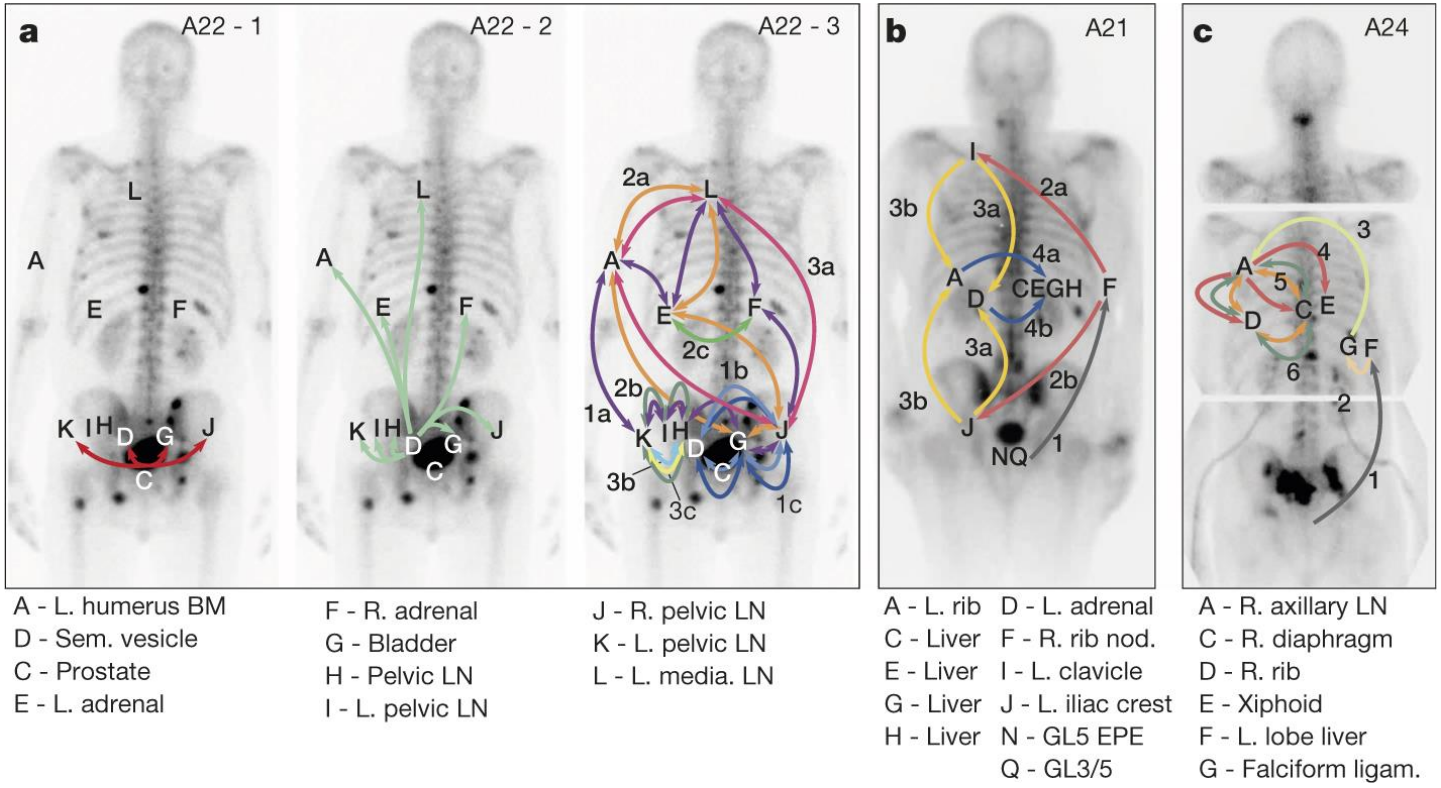


Hallmarks of cancer



- 90% of cancer related deaths are due to metastasis
- What kind of cells drives metastasis?

Metastasis-to-metastasis seeding occurs either by a linear or by a branching pattern of spread.



G Gundem *et al. Nature*, E1-E5 (2015) doi:10.1038/nature14347

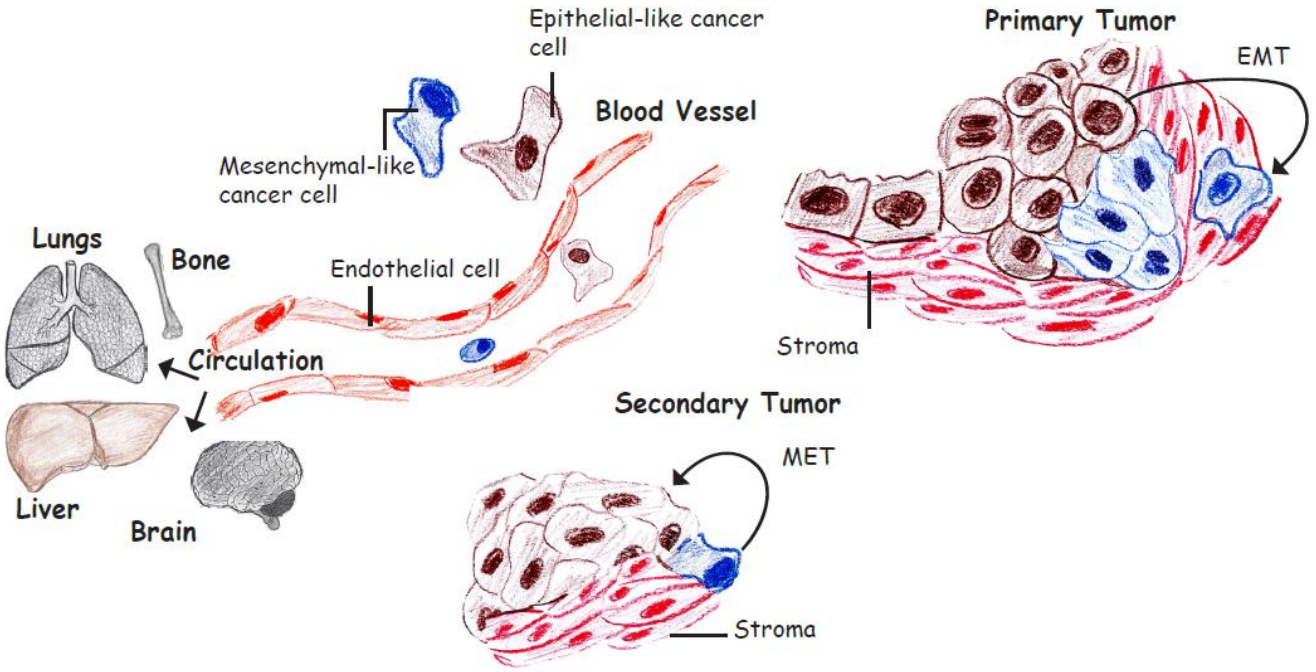
Hallmarks of metastasis-initiating cells

Characterized by evolutionary advantageous traits that may originate in primary tumor and continue to evolve during dissemination & colonization:

- **cellular plasticity**
- metabolic reprogramming
- ability to enter/exit dormancy
- immune evasion
- co-option of other tumor and stromal cells

Epithelial-to-mesenchymal transition (EMT)

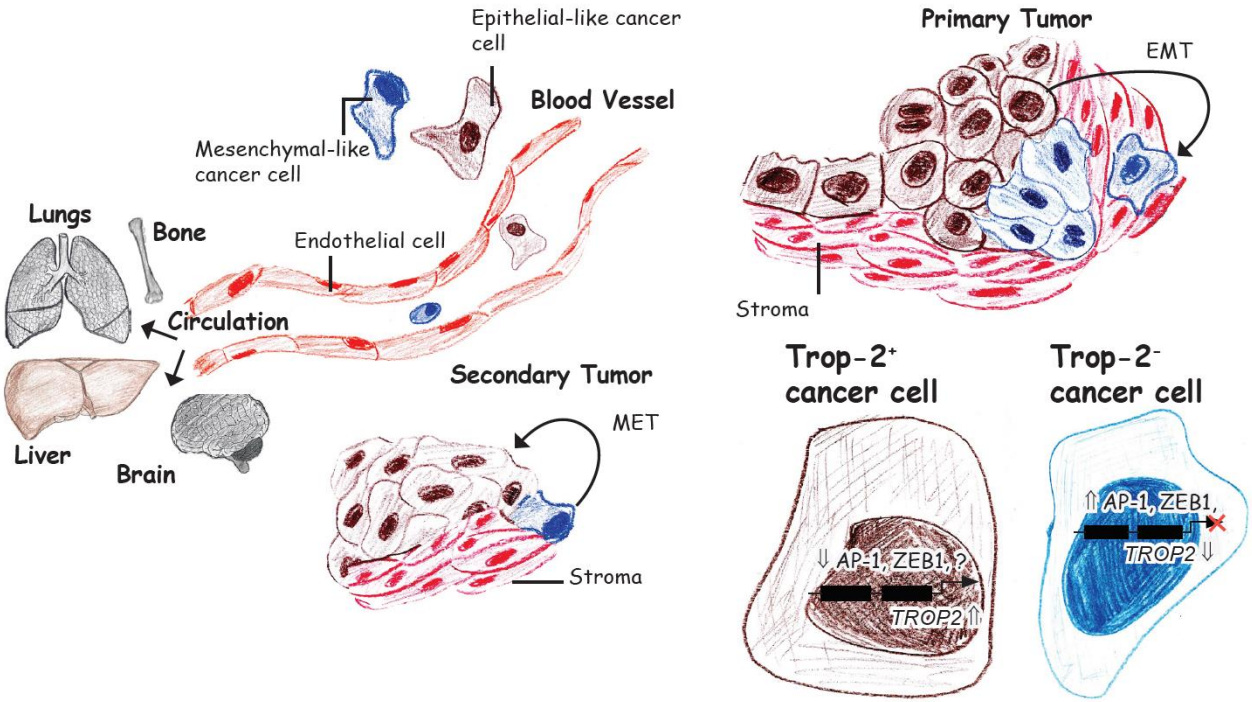
- Reversible acquisition of migratory and invasive properties by epithelial cells
- Role in embryonic development, fibrosis, cancer
- Creates cells with **stem-like cells** characteristics
- Both mesenchymal and epithelial phenotypes are required for efficient meta



Questions

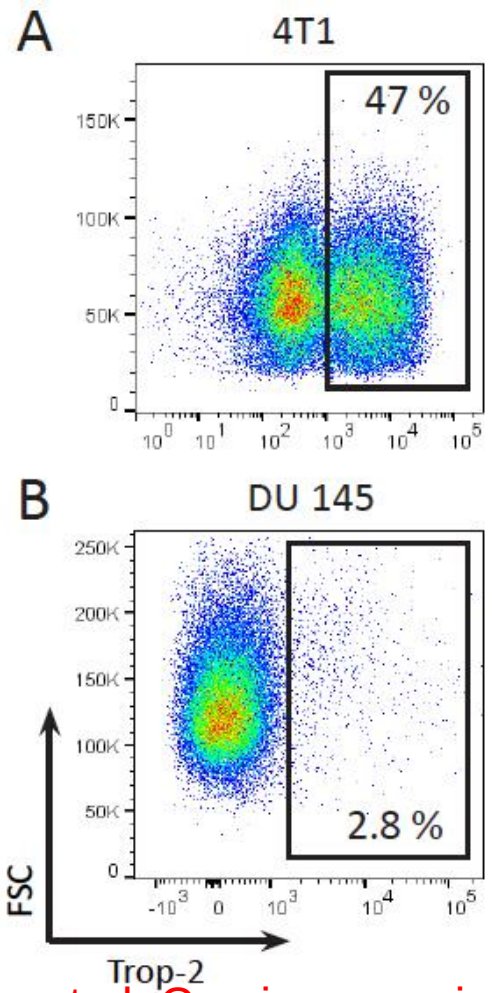
- What is a role of EMT in prostate and breast cancer progression?
  - Trop-2 associates with epithelial phenotype of breast and prostate cancer cells
  - EMT & metastatic signature of selected BCa subpopulations
- What is a role of cancer plasticity and heterogeneity in therapy resistance?
  - Synthetic lethality as a concept for treatment drug resistant cancer

Trop-2 associates with an epithelial phenotype in breast and prostate cancer cells



Trop-2 marks epithelial subpopulation of BCa and PCa cell lines

Hypothesis: EMT is accompanied by changes in CSC-like signature



Trop-2

- cell surface glycoprotein
- marks stem cells and progenitors
- role in stemness and multipotency maintenance mostly unknown, e.g. in prostate activates basal cell program
- in some tissues, expression is epigenetically silenced in differentiated adult cells
- described as oncogene and metastasis inductor

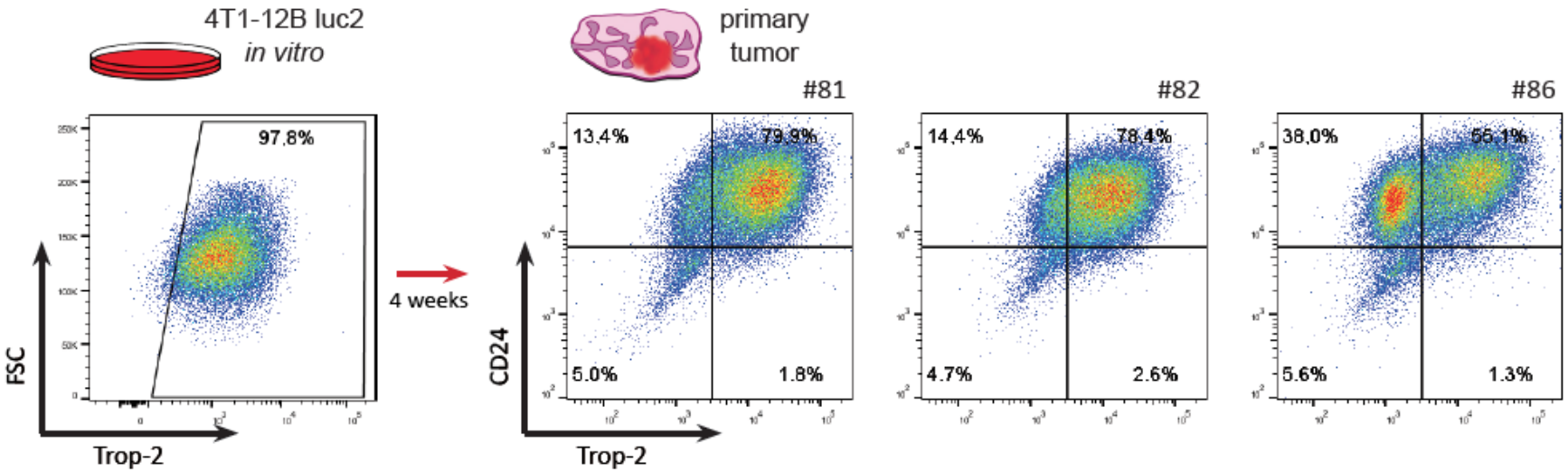
EpCAM vs. Trop-2

- both are commonly (over)expressed in adenoCa
- both are processed via RIP
- 67 % similarity
- 46 % promoter seq identity => quite unrelated
- EpCAM is known to be downregulated during EMT; but what about Trop-2?
- EpCAM KO has lethal phenotype

Remsik, et al, Carcinogenesis 2018

Dynamics of Trop-2 expression *in vivo & in vitro*

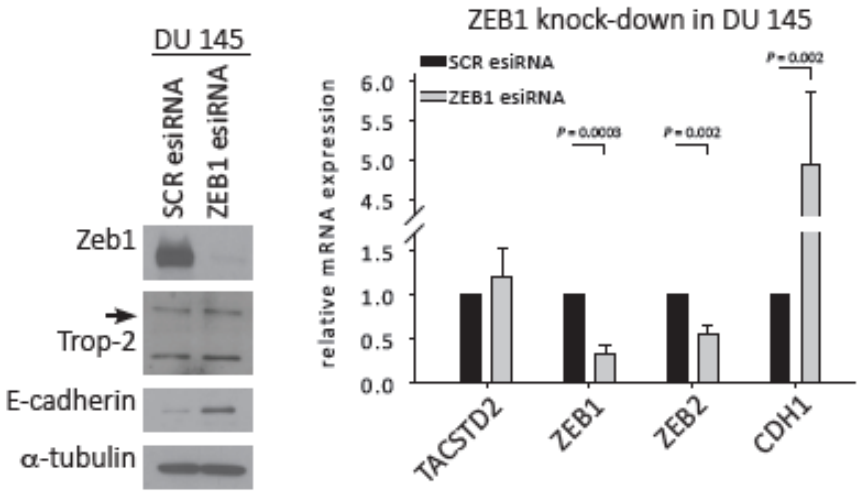
Hypothesis: Trop-2 is dynamically regulated and reflects epithelial state of cells



Remsik, et al, Carcinogenesis 2018

Regulation of Trop-2 expression by epigenetic and EMT machinery

Hypothesis: ZEB1 & DNA methylation regulate Trop-2

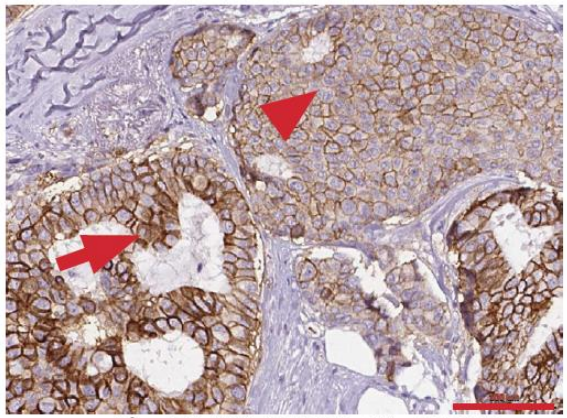


Remsik, et al, Carcinogenesis 2018

Intratumoral heterogeneity of membrane Trop-2 expression

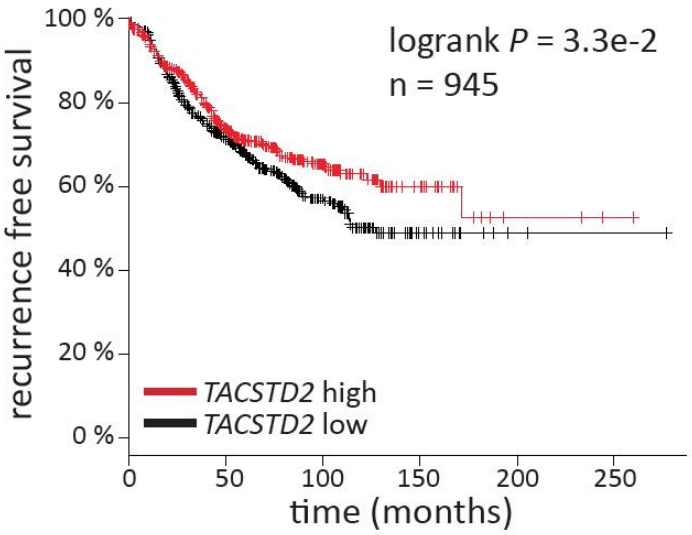
A

breast adenocarcinoma



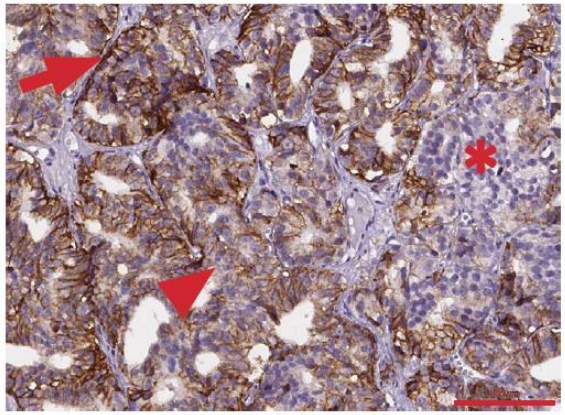
mAb: CAB072852, ID: 2428

B



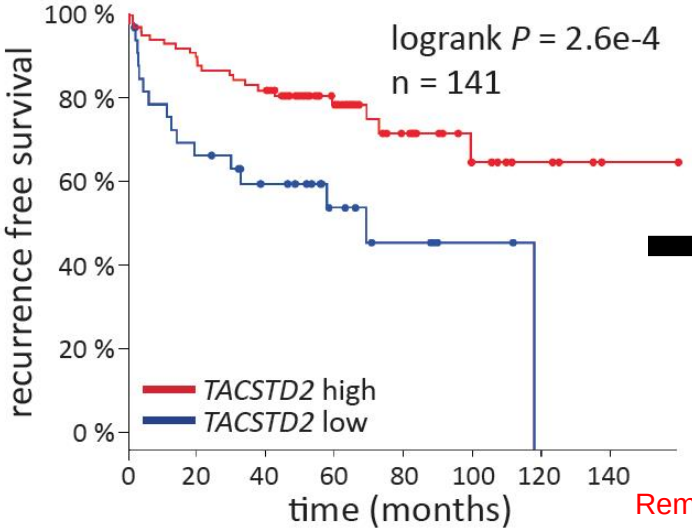
C

prostate adenocarcinoma



mAb: CAB072852, ID: 2816

D



→ better prognosis associated with epithelial phenotype

Remsik, et al, Carcinogenesis 2018

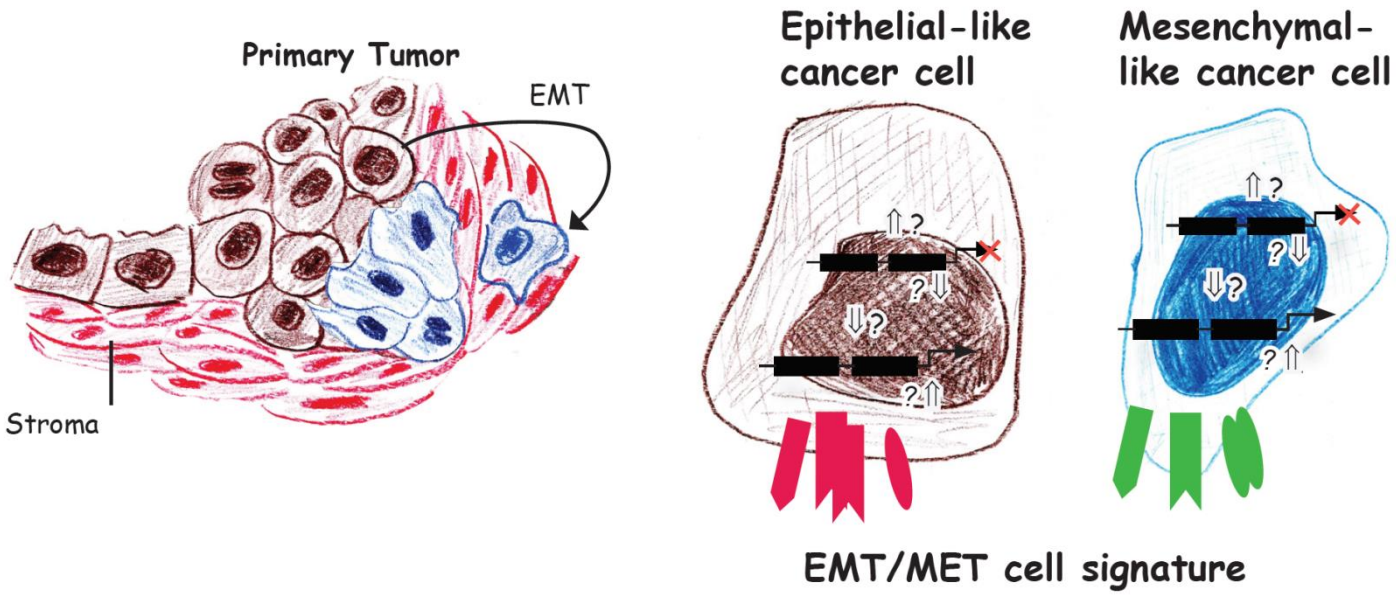
Summary

- Trop-2 associates with epithelial phenotype of breast and prostate cancer cells
- commonly accepted view of Trop-2 as oncogene is too simplistic

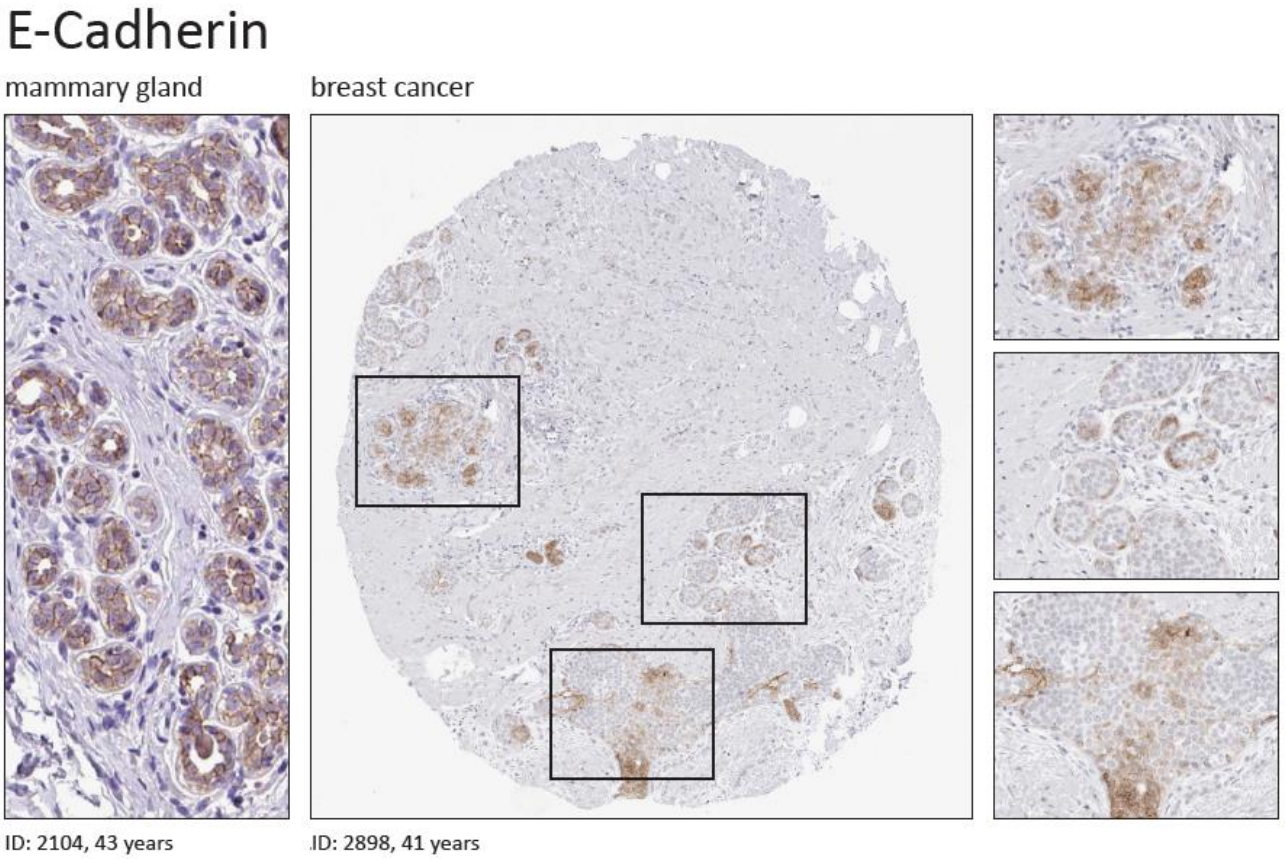
Future plans

- To uncover molecular mechanisms by which Trop-2 contributes to cancer progression
  - single cell qPCR in CTCs

EMT & metastatic signature of selected BCa subpopulations



Intratumoral heterogeneity and plasticity of cancer cells



EMT is commonly accepted source of plasticity, characterized by e.g. Cadherin-switch and often accompanied by activation of stem-like transcriptional programs

IHC source: www.proteinatlas.org

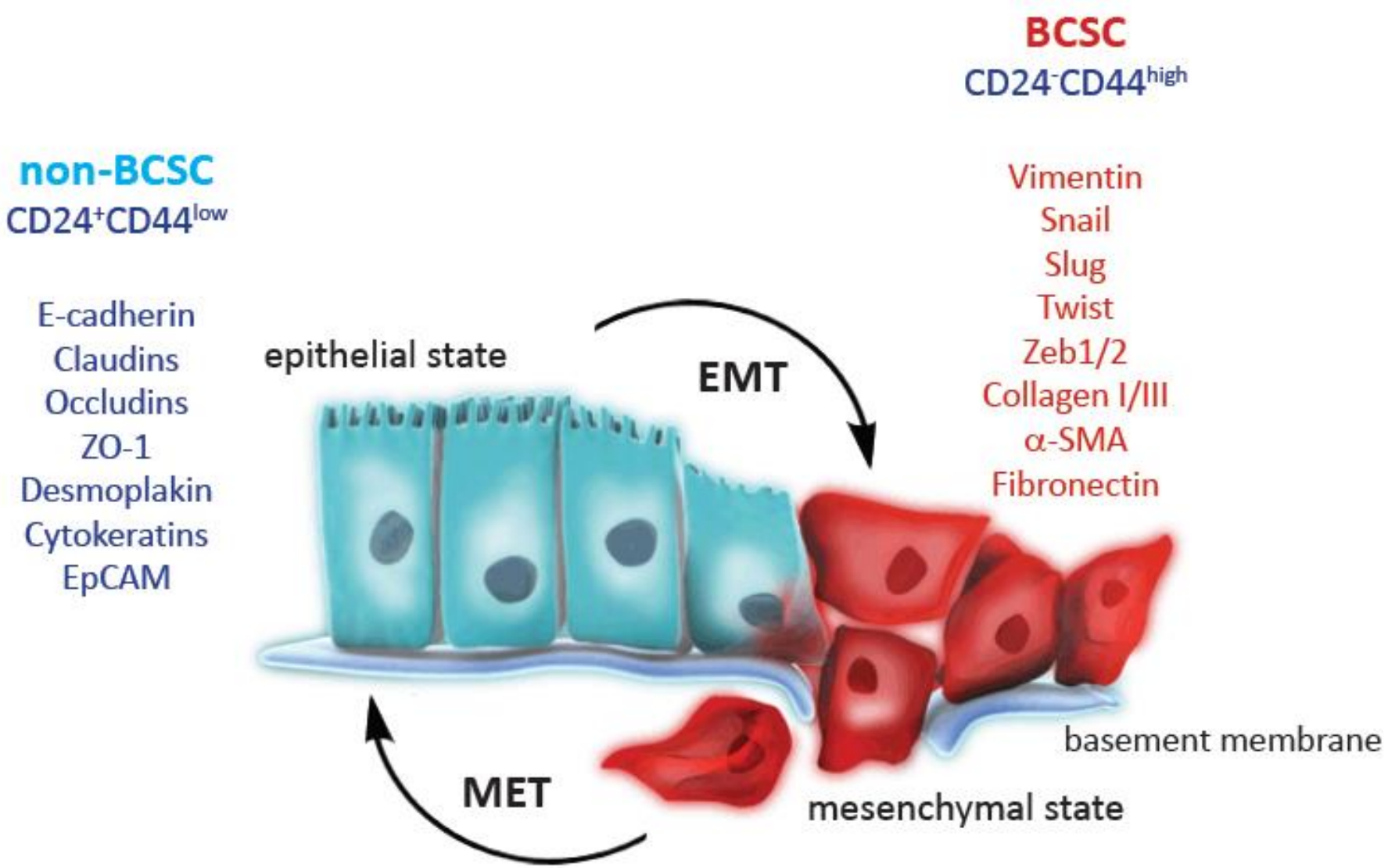
Motivation

TNBC is:

- Estrogen receptor (ER)-negative
- Progesteron (PR)-negative
- HER2 negative

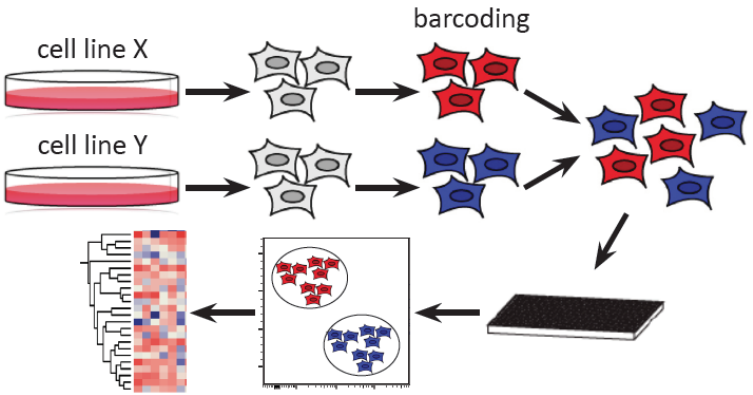
- 15-20% BCa
- Often in younger women, BRCA1 gene mutation
- Tends to be more aggressive, recur early and spread to other parts of body, poor prognosis
- treatment: surgery, radiation, chemotherapy (platinum-based, taxanes) = <u>no targeted therapy available</u>

Proteins associated with distinct cancer cell phenotypes

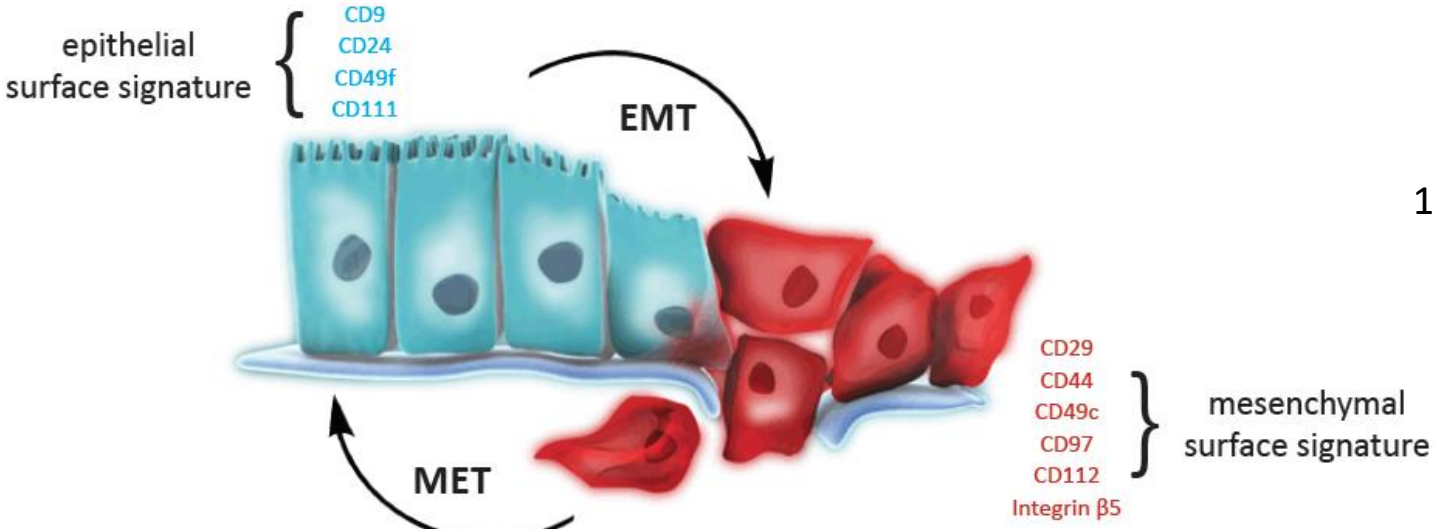


Surface antigens associated with distinct cancer cell phenotypes

Hypothesis: EMT-ed cells have specific surface pattern



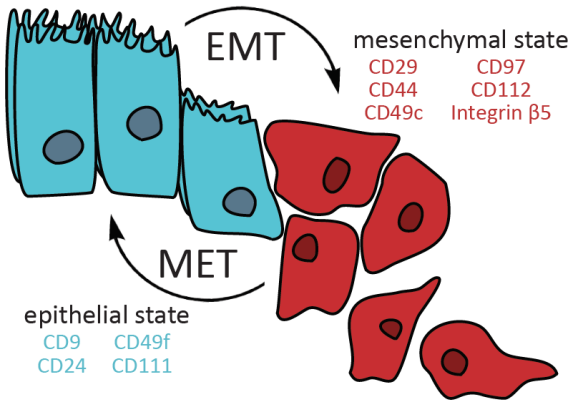
Surface marker	Gene	CAFTD03 x BPH-1	HMLE-EMT x HMLE	MCF10A-V12 x MCF10A
		[fold change]		
CD9	*CD9*	-1,661	-5,651	-1,427
CD24	*CD24*	-6,272	-5,881	-1,537
CD29	*ITGB1*	2,402	1,330	1,563
CD44	*CD44*	2,594	26,202	16,926
CD49c	*ITGA3*	1,722	1,671	2,154
CD49f	*ITGA6*	-1,507	-2,087	-1,426
CD97	*CD97*	1,454	2,060	1,659
CD111	*PVRL1*	-2,398	-2,015	-1,427
CD112	*PVRL2*	1,455	1,363	2,445
Integrin β5	*ITGB5*	2,837	3,358	3,110



next step:
11-colour FACS protocol
for patient samples

Remsik et al, BJC, 2018

Predicted 10-molecule surface signature that associates with plasticity of epithelial cells.



SHORT COMMUNICATION

BJC

British Journal of Cancer (2018), 1–7 | doi: 10.1038/bjc.2017.497

Keywords: breast cancer; epithelial-mesenchymal plasticity; intratumoural heterogeneity; CD9

Plasticity and intratumoural heterogeneity of cell surface antigen expression in breast cancer

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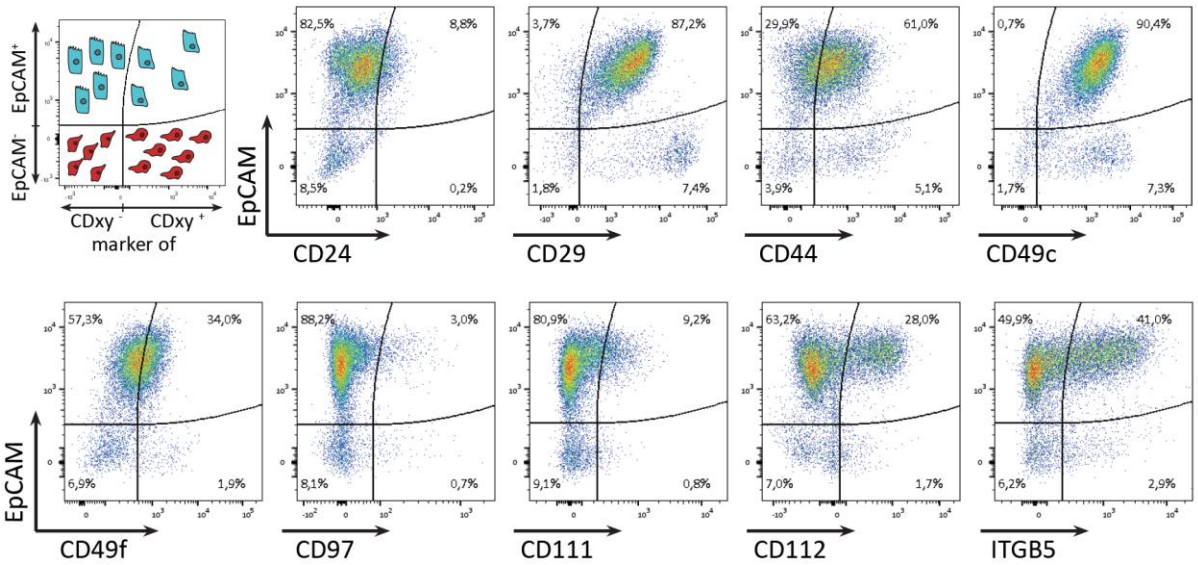
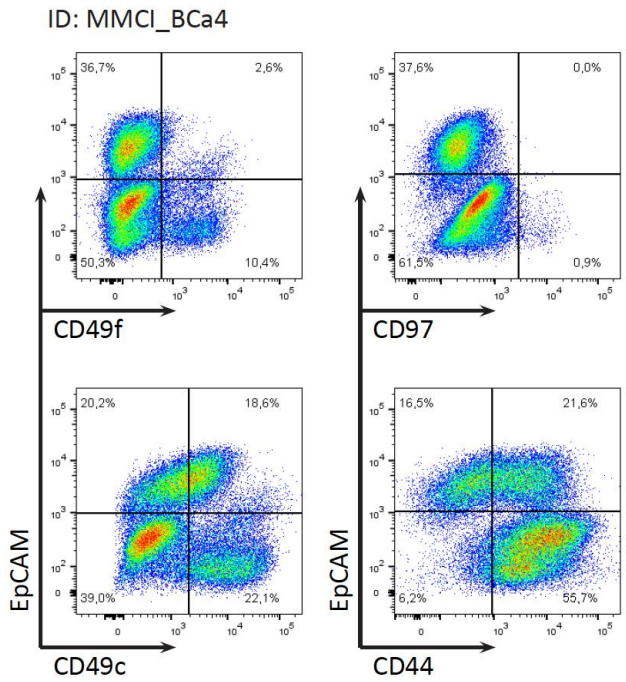
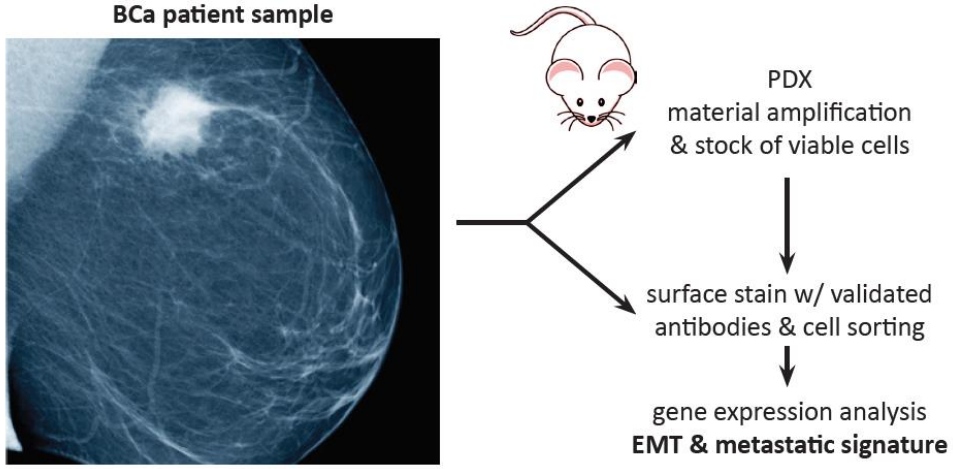


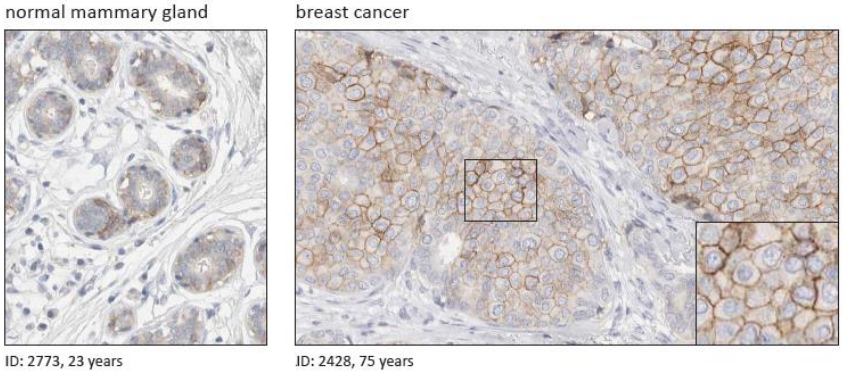
Heterogeneity examples



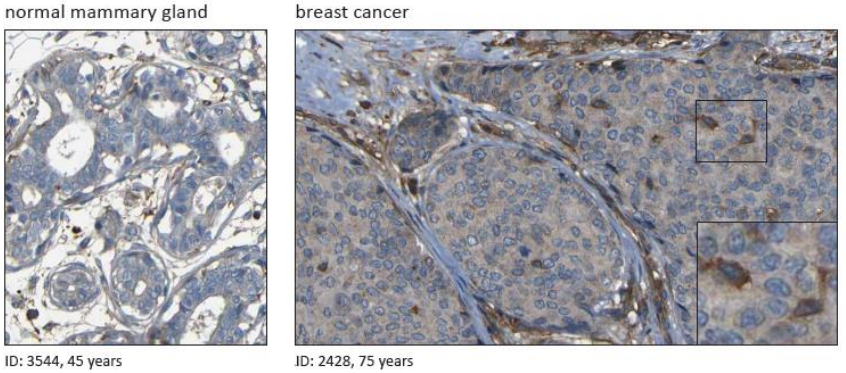
Experimental strategy



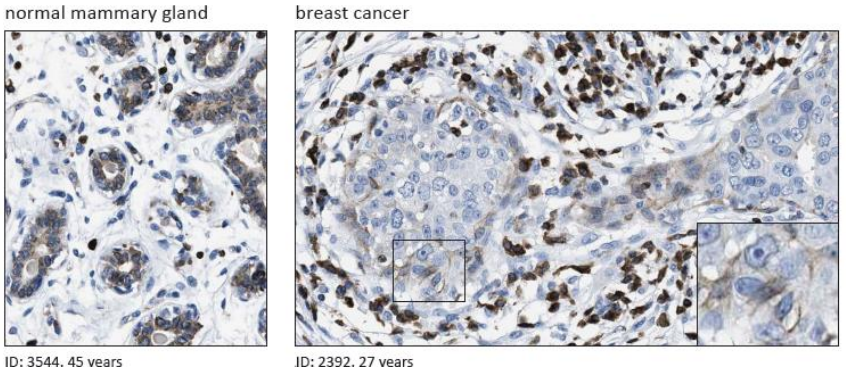
EpCAM



CD97



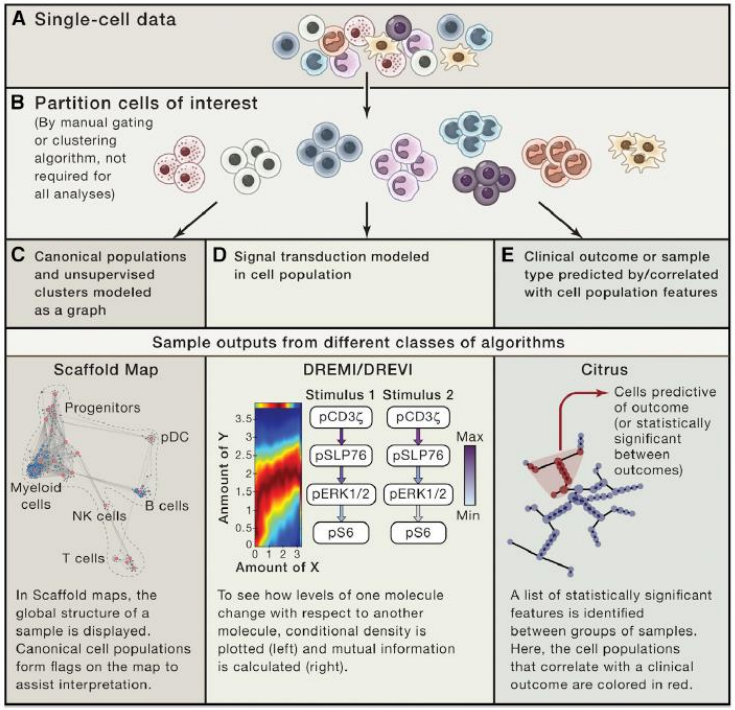
CD49c

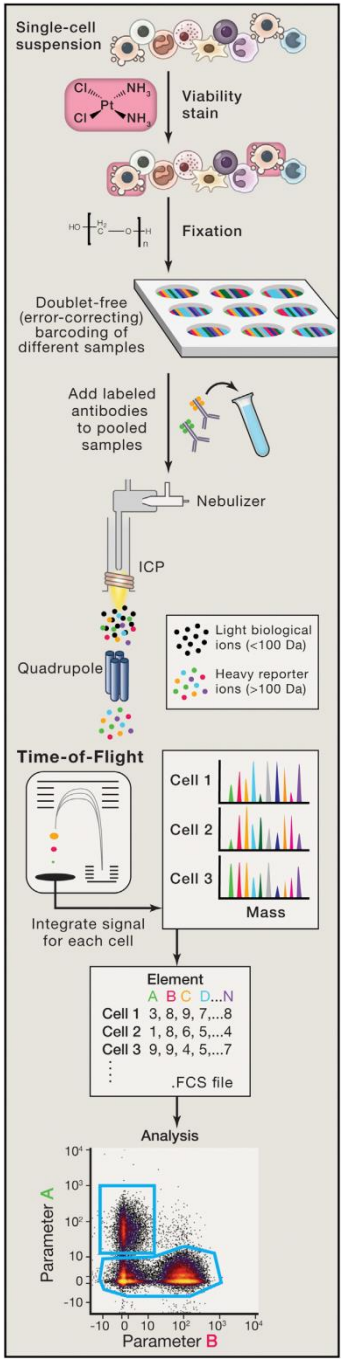


source: www.proteinatlas.org, www.fda.gov

Mass cytometry

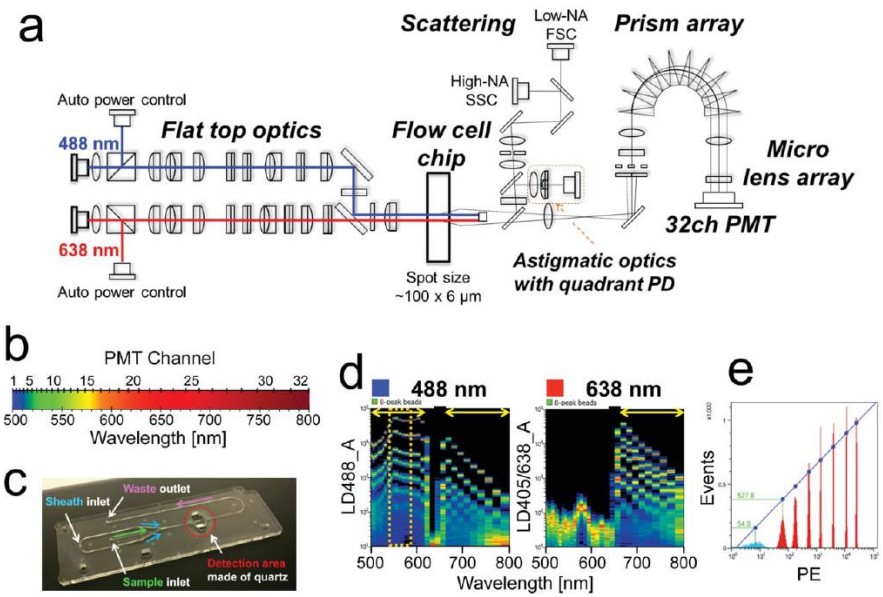
- measurement of over 40 simultaneous cellular parameters at single-cell resolution

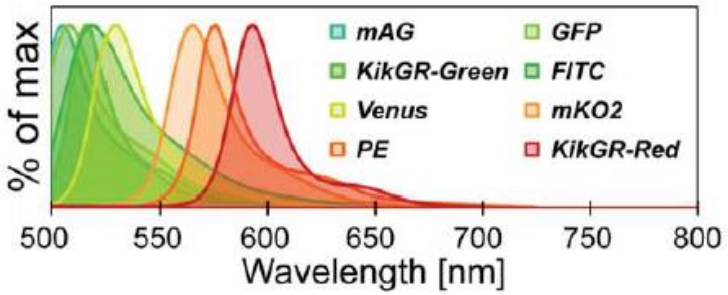


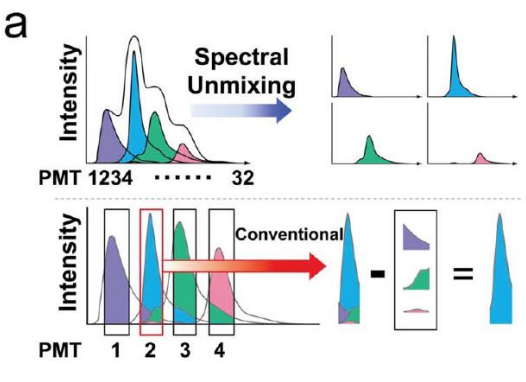


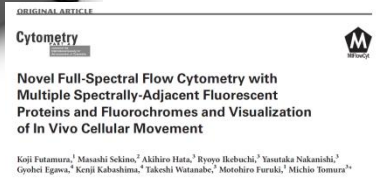
Cell *165*, May 5, 2016

Alternative … spectral FCM









Summary

- epithelial and stromal compartment of breast cancer tissue is composed of extremely heterogeneous mixture of cells

Future plans

- To identify cell surface signature which reflects cancer cell plasticity and mirrors enrichment in metastasis-initiating genes in patiens.
  - sample collection (actually various subpopulations from 3 patients sorted), processing and analysis
  - *in vitro* and *in vivo* tests for relevant markers (analysis of selected signaling pathways, migration, invasion)

Outlook

- Small-molecule drugs and synthetic lethality
- Plasticity of cancer cells and new targets for cancer therapy
- Modulation of tissue microenvironment, cell metabolism and drug efficiency

Team

- Ximena Muresan, Tereza Suchanková
- Stanislav Drápela, Ondřej Vacek, Markéta Pícková, Barbora Kvokáčková, Karolína Kryštofová
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- Department of Cytokinetics, Institute of Biophysics AS

Cooperation

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- Jan Bouchal, Gvantsa Kharaisvili - **UJP Olomouc**
- Jiří Kohoutek - **Veterinary Research Institute, Brno**
- Zoran Culig laboratory - **Medical University Innsbruck**
- Gabri Van der Pluijm - **Leiden University Medical Centre**
- Wytske van Weerden – **Erasmus University, NL**
- Michael Andäng, **Karolinska Institutet**
- Giuseppe Valachi**, University of Ferrara**