The role of cancer-associated fibroblasts and their extracellular vesicles in prostate cancer progression

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Introduction

- interaction between cancer cells and cancer-associated fibroblasts (CAFs) in the tumor microenvironment: important role for prostate cancer (PCa) development and progression
- mediated – besides other soluble factors and direct cell-cell contact – by the mutual exchange of extracellular vesicles (EVs; Fig. 1)
- aim of this project: analyze the functional effect of CAFs and their EVs on PCa cells *in vitro* and *in vivo*

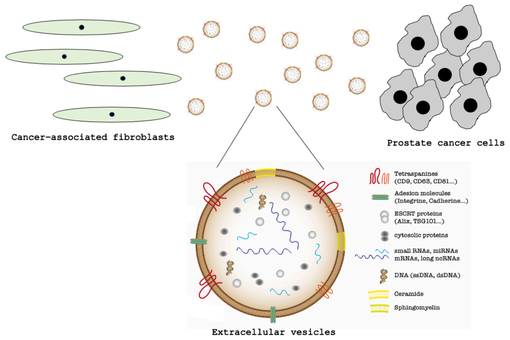

Fig. 1: Interaction of PCa cells and CAFs by the mutual exchange of EVs. PCa cells secreted EVs which are then taken up by CAFs in the tumor microenvironment and vice versa to induce biological changes in their target cells. EVs contain various biologically active molecules like DNA, mRNA, miRNA, proteins and lipids. EVs are characterized by certain soluble and membrane proteins reflecting their mode of biogenesis like Tetraspanins or ESCRT proteins (here: small EVs of endosomal origin). CD = cluster of differentiation, ESCRT = endosomal sorting complex required for transport.
modified from Linxweiler and Junker, Nat Rev Urol 2019

Materials & Methods

- establishment of primary cultures of cancer-associated (CAFs), not-cancer-associated (NCAFs) and benign prostate hyperplasia-associated (BPHFs) from human tissue samples
- characterization of fibroblast primary cultures by immunofluorescence staining (Pan-CK, Vimentin, αSMA)
- effect of fibroblasts on proliferation of PCa cells (LuCaP136 spheroids LNCaP cells): MTS assay
- coinjection of fibroblasts and PCa cells (5x10^5 each) in an orthotopic xenograft model → monitoring of tumor burden for 10 weeks by serum PSA measurements and small animal imaging
- isolation of fibroblast-secreted EVs by ultracentrifugation; characterization by Western Blot (WB), Nanoparticle Tracking analysis (NTA) and transmission electron microscopy (TEM)
- uptake of fibroblast-secreted EVs by PCa cells: *in vitro* fluorescence labeling experiments

Results: establishment and characterization of fibroblast primary cultures

- CAF, NCAF and BPHF primary cultures successfully established and characterized by IF (all Pan-CK negative, Vimentin positive; only CAFs αSMA positive)
- stable growth for up to 30 passages; use in further experiments: ≤ passage 10

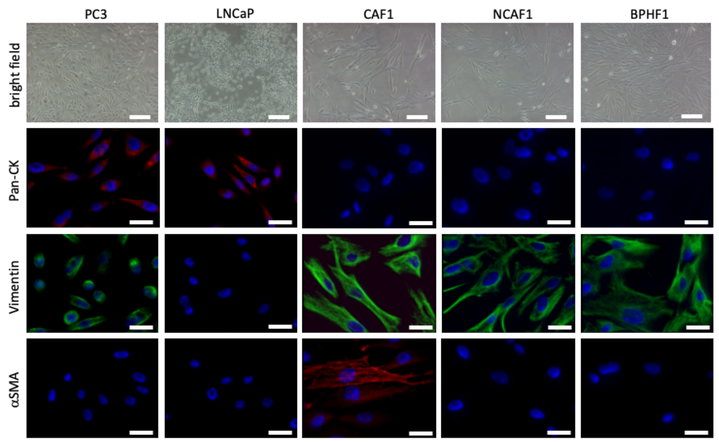

Fig. 2: Characterization of prostate fibroblast primary cultures. αSMA = alpha smooth muscle actin, CK = cytokeratin, scale bar (bright field) = 50μm, scale bar (immunofluorescence) = 20μm

effect of fibroblasts on PCa progression: *in vitro*

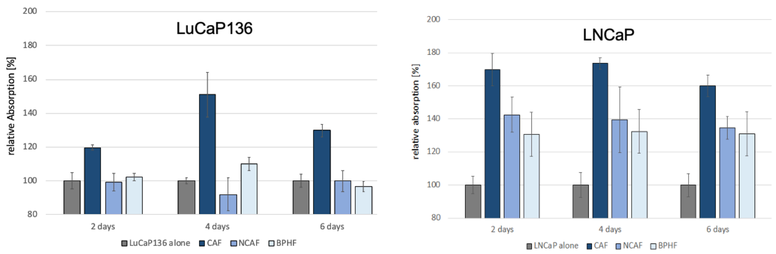

Fig. 3: Stimulation of PCa cell proliferation *in vitro*. Proliferation of LuCaP136 spheroids was stimulated only by CAFs, that of LNCaP cells by all three fibroblast types with CAFs showing the strongest effects. Means and standard deviations of three biological replicates are shown. Experiments were repeated with three different sets of CAF/NCAF/BPHF primary cultures, each with results comparable to that shown here.

effect of fibroblasts on PCa progression: *in vivo*

- orthotopic coinjection on PCa cells (LuCaP136, LNCaP) and fibroblasts (CAFs, NCAFs, BPHFs) in immunodeficient mice → 6 groups, n=8 mice per group
- stimulation of tumor growth and metastatic spread to lymph nodes and lungs by fibroblasts (CAFs showing strongest effect); differential effect in LuCaP136 and LNCaP
- Fig. 4, 5 and 6 and Tab. 1 show the results of the first *in vivo* experiment, which was then repeated two times with other sets of fibroblast primary cultures, with same results

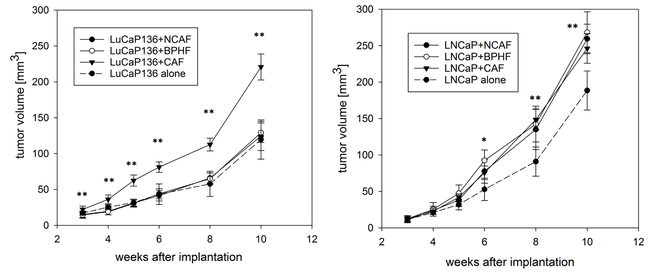

Fig. 4: Primary tumor growth after orthotopic coinjection of PCa cells and fibroblasts. Tumor volumes were determined by high-resolution 3D ultrasonography. Primary tumor growth was significantly stimulated by coinjection with CAFs (LuCaP136 xenografts) and with all fibroblasts (LNCaP xenografts) compared to the injection of PCa cells alone. Serum-PSA reflected well the differences seen in primary tumor volumes (data not shown). ** p<0.01

Tab. 1: Development of lymph node and lung metastases after coinjection with different fibroblast primary cultures in orthotopic LuCaP136 and LNCaP xenografts. 10 weeks after intraprostatic injection of 5x10^5 LuCaP136 or LNCaP cells combined with 5x10^5 CAF, NCAF or BPHF cells, mice were sacrificed and their organs were examined for the presence of metastases. The number of animals with lymph node and lung metastases in each of the 6 resulting groups is given here. Single animals in different combination groups (initially n=8 in both groups) died prematurely due to not cancer-specific causes. p-values were determined by Fisher´s exact test.

	≥ 2 LN metastases	statistical significance	lung metastases	statistical significance
LuCaP136 alone (n=6)	2/6	/	0/6	/
LuCaP136 + NCAF1 (n=6)	1/6	/	0/6	/
LuCaP136 + BPHF1 (n=7)	2/7	p = 1,00 (vs. LuCaP136 alone) p = 0,63 (vs. LuCaP136 + NCAF1)	1/7	p = 1,00 (vs. LuCaP136 alone) p = 1,00 (vs. LuCaP136 + NCAF1)
LuCaP136 + CAF1 (n=7)	6/7	p = 0,10 (vs. LuCaP136 alone) **p = 0,03 (vs. LuCaP136 + NCAF1)** p = 0,10 (vs. LuCaP136 + BPHF1)	4/7	p = 0,07 (vs. LuCaP136 + NCAF1) p = 0,07 (vs. LuCaP136 + NCAF1) p = 0,27 (vs. LuCaP136 + BPHF1)
LNCaP alone (n=7)	1/7	/	1/7	/
LNCaP + NCAF1 (n=7)	1/7	/	2/7	/
LNCaP + BPHF1 (n=7)	2/7	p = 1,00 (vs. LNCaP alone) p = 1,00 (vs. LNCaP + NCAF1)	2/7	p = 1,00 (vs. LNCaP alone) p = 1,00 (vs. LNCaP + NCAF1)
LNCaP + CAF1 (n=7)	4/7	p = 0,27 (vs. LNCaP alone) p = 0,27 (vs. LNCaP + NCAF1) p = 0,59 (vs. LNCaP + BPHF1)	5/7	p = 0,10 (vs. LNCaP alone) p = 0,29 (vs. LNCaP + NCAF1) p = 0,29 (vs. LNCaP + BPHF1)

isolation and characterization of fibroblast-secreted EVs

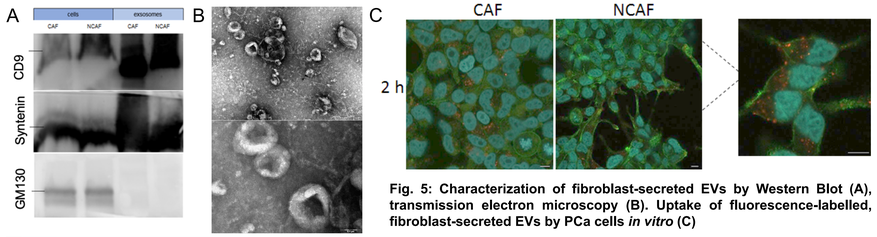

Fig. 5: Characterization of fibroblast-secreted EVs by Western Blot (A), transmission electron microscopy (B). Uptake of fluorescence-labelled, fibroblast-secreted EVs by PCa cells *in vitro* (C)

Conclusions

- successful isolation and characterization of prostate fibroblast primary cultures and their EVs
- CAFs stimulate the growth of PCa cells *in vitro* and *in vivo*
- fibroblast-secreted EVs are efficiently taken up by PCa cells
- the molecular mechanisms involved in PCa cell <> fibroblast interaction, especially the role of EVs, will be further elucidated in future work

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