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, α SMA)
- effect of fibroblasts on proliferation of PCa cells (LuCaP136 spheroids LNCaP cells): MTS assay
- coinjection of fibroblasts and PCa cells (5x10⁵ each) in an orthotopic xenograft model \rightarrow 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 α SMA positive)
- stable growth for up to 30 passages; use in further experiments: \leq passage 10



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

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 \rightarrow 6 groups, n=8 mice per group
- stimulation of tumor growth and metastatic spread to lymph nodes and lungs by
- Fig. 4, 5 and 6 and Tab. 1 show the results of the first *in vivo* experiment, which was





fibroblasts (CAFs showing strongest effect); differential effect in LuCaP136 and LNCaP then repeated two times with other sets of fibroblast primary cultures, with same results



LuCaP136 alone (n=6) Tab. 1: Development of lymph node and lung metastases after coinjection with LuCaP136 1/6 0/6 different fibroblast primary cultures in + NCAF1 (n=6) LuCaP136 LNCaF orthotopic and p = 1,00 (vs. LuCaP136 alone) LuCaP136 p = 1,00 (vs. LuCaP136 alone) xenografts. 10 weeks after intraprostatic p = 0,63 (vs. LuCaP136 + NCAF1) p = 1,00 (vs. LuCaP136 + NCAF1) + BPHF1 (n=7) injection of 5x10⁵ LuCaP136 or LNCaP cells combined with 5x10⁵ CAF, NCAF or BPHF p = 0,07 (vs. LuCaP136 alone) p = 0,10 (vs. LuCaP136 alone) LuCaP136 cells, mice were sacrificed and their organs p = 0,07 (vs. LuCaP136 + NCAF1) = 0,03 (vs. LuCaP136 + NCAF1 + CAF1 (n=7) for the presence of were examined p = 0,10 (vs. LuCaP136 + BPHF1) p = 0,27 (vs. LuCaP136 + BPHF1) metastases. The number of animals with lymph node and lung metastases in each of the 6 LNCaP alone resulting groups is given here. Single animals (n=7) in different combination groups (initially n=8 in LNCaP both groups) died prematurely due to not + NCAF1 (n=7) cancer-specific causes. p-values were determined by Fisher's exact test. LNCaP p = 1,00 (vs. LNCaP alone) p = 1,00 (vs. LNCaP alone) p = 1,00 (vs. LNCaP + NCAF1) p = 1,00 (vs. LNCaP + NCAF1) + BPHF1 (n=7) p = 0,10 (vs. LNCaP alone) p = 0,27 (vs. LNCaP alone) LNCaP p = 0,29 (vs. LNCaP + NCAF1) p = 0,27 (vs. LNCaP + NCAF1 + CAF1 (n=7) p = 0,29 (vs. LNCaP + BPHF1) p = 0,59 (vs. LNCaP + BPHF1)

isolation and characterization of fibroblast-secreted EVs





Conclusions

- EVs, will be further elucidated in future work

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Fig. 4: Primary tumor growth after orthotopic coinjection of PCa cells and fibroblasts. Tumor volumes were by high-resolution 3D ultrasonography. Primary tumor growth stimulated by CAFs (LuCaP136 with all fibroblasts with (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



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)

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

B. BRAUN-STIFTUNG DER GESUNDHEIT NEUE WEGE BEREITEN



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