57 Fibroblast-secreted exosomes in prostate cancer

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Introduction & Objectives: The crosstalk between prostate cancer (PCa) cells and cancer-associated fibroblasts (CAFs), is – among others - mediated by the exchange of exosomes. In this project, the role of CAFs and their secreted exosomes for tumor-stroma interaction in PCa is being investigated to gain new insights into the molecular mechanisms associated with local and systemic PCa progression.

Materials & Methods: Cancer-associated (CAFs) and not-cancer-associated fibroblasts (NCAFs) were cultivated from primary PCa samples obtained immediately after surgery. Furthermore, benign prostate hyperplasia associated fibroblasts (BPHFs) were grown from BPH tissue samples.Immunofluorescence staining (α-SMA, cytokeratin, vimentin) was performed to characterize the primary cultures. The influence of primary fibroblasts on the viability of PCa cells (LuCaP136, LNCaP) was investigated by co-cultivation for up to 6 days and subsequent MTS assays. Isolation of fibroblast-secreted exosomes was performed by ultracentrifugation. Quality of isolated exosomes was examined by Western Blot and ExoView analysis (CD9, CD63, Syntenin, GM130). The transfer of exosomes from fibroblasts to PCa cells (LNCaP, C4-2) was investigated by 2/6/12-hour incubation of PCa cells with PKH26-labelled CAF- and NACF-exosomes and subsequent fluorescence microscopy.

Results: Using a specific protocol we are able to obtain stably growing CAF, NCAF and BPHF primary cultures. Immunostaining showed negative cytokeratin and positive vimentin signals proving the mesenchymal origin of the fibroblasts. α-SMA stained in CAFs only with 10 to 50% of cells being positive. Cocultivation with CAFs significantly stimulated the viability of LuCaP136 spheroids compared to cocultivation with NCAFs and BPHFs. In contrast, the viability of LNCaP cells was significantly promoted by all three fibroblast types, while CAFs induced the strongest effect. Exosomes could be successfully isolated by ultracentrifugation from CAF and NCAF conditioned media. Western Blot and ExoView analyses showed the expression of typical exosomal markers (CD9, CD63, Syntenin) and the absence of the cellular contamination marker GM130. Transfer of exosomes to Pca cells could be demonstrated with the strongest IF signals obtained after 2h incubation.

Conclusions: Both, cultivation of primary prostate fibroblasts and isolation of their secreted exosomes could be successfully established. It could be shown that fibroblast-secreted exosomes are taken up and processed by PCa cells in a time-dependent manner. Fibroblasts and especially CAFs significantly stimulated the viability of PCa cells. Future studies will examine the effect of prostate fibroblasts and their exosomes on further traits of PCa cells like migration and invasion potential.