Reprogramming CAFs for tumor killing: a gene therapy approach

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Abstract:
Due to their proximity to the tumor blood vessels and their abundance in the tumor stroma, Cancer Associated Fibroblasts (CAFs) have emerged as a new appealing target for gene therapy approaches aimed at the in situ production of tumor selective cytotoxic agents, such as TRAIL (TNF-related apoptosis-inducing ligand).

During my master thesis I worked on the validation that – independently on the gene therapy vector – CAFs can be used as a "depot" of TRAIL, which should trigger cancer cells apoptosis through an exosomes-mediated mechanism. The main objectives of my work were: the optimization of TRAIL production by CAFs and the evaluation of TRAIL vesicular localization.

To resemble CAFs population we treated NIH3T3 fibroblasts with TGF-β (Transforming Growth Factor beta). To optimise TRAIL production by CAFs we compared pDNA transfection and mRNA transfection. In both cases of pDNA and mRNA TRAIL was fused to the fluorescent tag EGFP (Enhanced Green Fluorescent Protein), we evaluated the transfection efficiency by flow cytometry. By the same technique we also assayed transfection-associated toxicity. CAFs transfection studies demonstrated that mRNA transfection solved the problems of low transfection efficiency and high toxicity associated to pDNA transfection. For this reason, we selected the mRNA for the following studies. We then transfected 4T1 breast cancer cells and CAFs with EGFP and EGFP-TRAIL mRNAs and we evaluated the cellular apoptosis by the annexin V/DAPI assay. The results demonstrated that TRAIL triggers apoptosis selectively in cancer cells, by a bystander effect. To validate TRAIL localization in TRAIL$^+$ transfected CAFs-released exosomes we studied the fusion protein subcellular localization by confocal microscopy. In parallel we set up the experimental conditions for CAFs-derived exosomes isolation and Western Blot (WB) characterization. The WB results confirmed that the obtained sample contained exosomes as exosomes markers such as Lamp1 (Lysosomal-associated membrane protein 1), CD63 (Lysosomal-associated membrane protein 3), CD81 (Tetraspanin-28) and Tsg101 (Tumor susceptibility gene 101 protein) have been detected. However, the confocal studies revealed that the TRAIL fusion to EGFP tag prevented the protein from reaching the plasma membrane. As a matter of fact, EGFP-TRAIL has a cytoplasmic localization. For this reason, we decided to swich from EGFP-TRAIL mRNA to no tag TRAIL mRNA for the future studies.

As next step we will transfect CAFs with TRAIL mRNA and validate TRAIL localization by performing confocal microscopy studies on TRAIL-transfected CAFs and WB analysis on the exosomes isolated from TRAIL-transfected CAFs cellular medium. Then, we will investigate the apoptotic activity of TRAIL$^+$-exosomes in vitro.