Killing cancer cells by targeting the tumor microenvironment: study of TRAIL journey in a CAFs model

Baioni C.[1], Innocenti M.[1], Colombo M.[1], Prosperi D.[1]
E-mail: chiara.baioni@unimib.it
[1] NanoBioLab, Department of Biotechnology and Biosciences, University of Milano Bicocca (Italy)

Keywords: CAFs, TGFβ, TRAIL, exosomes

Abstract:

Tumor Necrosis Factor Related Apoptosis-Inducing Ligand (TRAIL) is a cytotoxic membrane protein physiologically expressed into the plasma membrane. It can be subsequently released in the extracellular milieu both as a membrane protein through exosomes and as a soluble factor, less active than the membrane-bound one. TRAIL has received great attention as a potential anticancer agent due to its ability to selectively induce apoptosis in cancer cells through a p53-independent mechanism. This led to the development of many TRAIL-receptors agonists which, however, failed to translate into clinics due to poor pharmacokinetics and weak apoptotic effects. A TRAIL-based gene therapy could offer a solution to these problems.

This project aims at giving a proof of concept that Cancer Associated Fibroblasts (CAFs) – the most accessible cells at the tumor microenvironment – can be selected as target cells of a gene therapy approach intended to produce TRAIL at the tumor stroma to kill cancer cells by exosomes.

For this purpose, we developed a CAFs model by treating NIH3T3 fibroblasts with Transforming Growth Factor β (TGFβ), the major driver of fibroblasts differentiation into the cancer-associated phenotype. We demonstrated the differentiation and its stability overtime by quantifying the expression of CAFs markers both at transcriptional and protein level (by qPCR and WB, respectively) and by analysing CAFs metabolic switching towards a glycolytic phenotype (by Seahorse). Then, we transfected NIH3T3/CAFs with TRAIL mRNA and we investigated TRAIL subcellular and extracellular localization by confocal microscopy and cell fractionation and by WB and ELISA, respectively. Our studies revealed that surprisingly TRAIL has a cytoplasmic localization into transfected cells, and that these cells partially release it into the extracellular milieu in association with exosomes. The future studies will be focused both on a deeper understating of TGFβ effects on NIH3T3 cells and on the investigation of the anticancer activity of $TRAIL^+$ vesicles.