TARGETING PANCREATIC DUCTAL ADENOCARCINOMA WITH A CHEMO & GENE THERAPY STRATEGY

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) shows an highly malignant phenotype with a poor response to currently available therapies (Malvezzi et al., 2014). A hallmark of this malignancy is the pronounced fibrotic stroma, composed by stromal cells, endothelial and inflammatory cells, collagen, cytokines, chemokines and growth factors stored in the extracellular matrix (ECM) (Swartz et al., 2012; Yu et al., 2012; Hartmann et al., 2014; Kota et al., 2017). Among these components, stromal cells represent the most abundant cellular subtypes in PDAC microenvironment and play a key role in tumor development and progression compromising the delivery of chemotherapeutics into neoplastic tissue and protecting tumor cells towards anticancer compounds. For these reasons, significant efforts have been recently focused on the development of novel therapies able to target both malignant and stromal cellular elements. Since years, we have developed an anti-tumor gene-therapy strategy based on human adipose mesenchymal stromal/stem cells (AD-MSC) engineered to secrete the proapoptotic soluble (s)-TRAIL protein (Spano et al., 2019; Rossignoli et al. 2019). By 2D and 3D culture models, we have investigated the cytotoxic impact obtained on PDAC environment combining both AD-MSC sTRAIL and chemotherapeutic drugs, such as Gemcitabine (Gem) and Nab-Paclitaxel (Nab-PTX).

Material and Methods

PDAC sample dissociation. After informed consent, four human PDAC samples have been collected from patients undergoing to surgery. Primary stromal cells were isolated by mechanical and enzymatic dissociation using Tumor Dissociation Kit and the gentleMACS Octo Dissociator (MACS Miltenyi Biotec). **Immunophenotype by FACS analysis.** Isolated stroma has been stained with anti-CD73 (PE; Miltenyi Biotec) and -CD90 (APC; BD Biosciences); anti-CD105 (FITC; BD Biosciences), -DcR1 (PE; Biolegend) and -DcR2 (APC; R&D Systems); anti-CD45 (FITC; BD Biosciences), -DR4 (PE; Biolegend) and -DR5 (APC; eBioscience); anti-EpCAM (PE; eBioscience) and -HLA-DR (FITC; eBioscience); PE (BD Biosciences), APC (Miltenyi Biotec) and FITC (BD Biosciences) isotype controls. Analyses have been performed by FACS Aria III (Becton Dickinson). **2D cytotoxicity assay.** PDAC stromal cells (5.000/well) have been seeded in 96 well plates. Cells have been treated with sTRAIL alone (from 50 to 2500pg/ml) for 24h or pre-treated with Gem (10uM) and Nab-PTX (1uM) for 24h and then with sTRAIL (1000pg/ml) for further 24h. Stromal cell viability has been evaluated by CellTiter Glo assay (Promega). **3D cytotoxicity assay.** Luciferase positive BXPC-3 cells (247.500/VITVO) and GFP positive stromal cells (202.500/VITVO) have been loaded into the small bioreactor VITVO® (Rigenerand S.r.l., Medolla, MO, Italy) (Candini et al., 2019). Co-culture has been pre-treated with Gem (10uM) and Nab-PTX (1uM) for 24h and then AD-MSC sTRAIL have been added at different E:T ratios (1:30 and 1:10; E = AD-MSC sTRAIL, T = BXPC-3). After 24, 48 and 72h tumor cell death has been quantified by bioluminescence signal decrease.

Results

❖ Isolation and characterization of stroma from four primary PDAC samples

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PRIMARY PDAC SAMPLE	Diagnosis	Size of tumor sample	N° of isolated cells
PZ1	Moderately differentiated ductal adenocarcinoma of pancreas	500 mg	1.925.000
PZ2	Moderately differentiated adenocarcinoma of pancreas	330 mg	238.000
PZ3	Moderately (70%) and poorly (30%) differentiated ductal adenocarcinoma of pancreas	330 mg	380.000
PZ4	Poorly differentiated adenocarcinoma of pancreas and of the wall and nerves of choledoco	38 mg	300.000

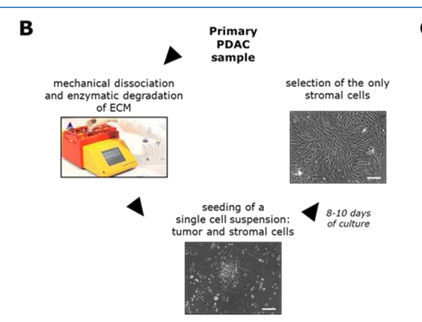

Figure 1. Primary PDAC stromal cells express CD73, CD90, CD105 and DR5. Stromal cells have been isolated from four primary PDAC samples (PZ1, PZ2, PZ3 and PZ4; Figure 1A) as represented in Figure 1B. They showed a typical mesenchymal immunophenotype, expressing CD73, CD90 and CD105 and lacking CD45, EPCAM and HLA-DR antigens (Figure 1C; mean of the four samples). Moreover, they revealed a negligible expression of TRAIL receptors except for DR5, suggesting that TRAIL-mediated apoptosis could be triggered by DR5 on this cell type (Figure 1C; mean of the four samples).

❖ Cytotoxic effect of Gem/Nab-PTX & sTRAIL on PDAC stroma in 2D

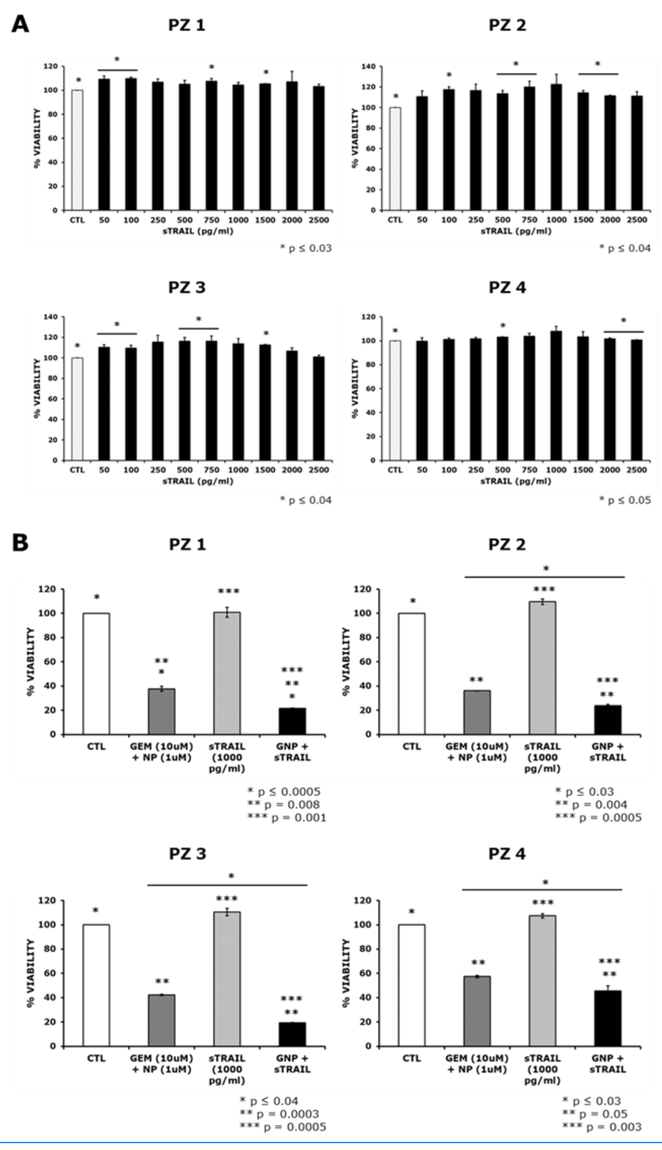

Figure 2. Gem/Nab-PTX co-treatment sensitizes PDAC stromal cells to sTRAIL in 2D. 2D dose response assay revealed that primary PDAC stromal cells were refractory to the proapoptotic effect mediated by sTRAIL alone for 24h (Figure 2A). In contrast, combinatory treatment with Gem/Nab-PTX & sTRAIL provoked a significant decrease of stromal cell viability compared to either sTRAIL alone or chemotherapeutic drugs alone (Figure 2B). These data suggest that the trio-combo approach displays a synergistic pro-apoptotic effect on PDAC stroma able to overcome the TRAIL resistance.

❖ Cytotoxic effect of Gem/Nab-PTX & AD-MSC sTRAIL on PDAC microenvironment in 3D

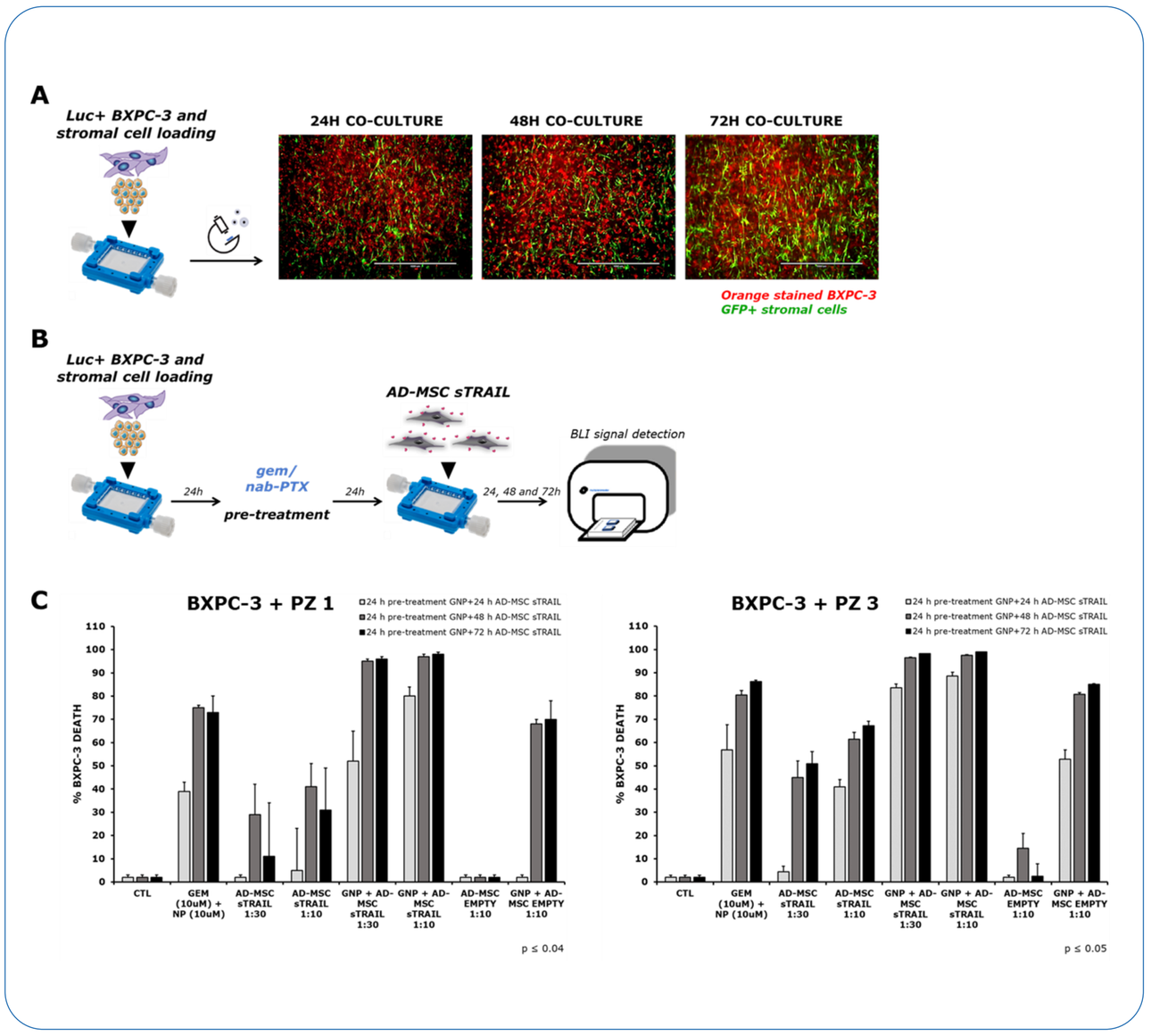

Figure 3. Synergistic impact of Gem/Nab-PTX & AD-MSC sTRAIL in a 3D PDAC model. A 3D co-culture, mimicking an *in vivo*-like PDAC model, was generated in a novel 3D bioreactor (VITVO®). BXPC-3 cells (red) and PDAC stromal cells (green) were co-cultured to recreate a tumor-like tissue *in vitro* (Figure 3A). 3D PDAC model was used to investigate the therapeutic impact of Gem/Nab-PTX & AD-MSC sTRAIL in a more reliable scenario than 2D culture. 3D PDAC was pre-treated with Gem/Nab-PTX for 24h, then AD-MSC sTRAIL were added at different E:T ratios (Figure 3B). For the three time points and both E:T ratios tested, the trio-combo treatment provoked a higher BXPC-3 cell death compared to single treatments (Figure 3C). Synergism of the chemo & AD-MSC sTRAIL therapy displays a significative antitumor effect on an *in vivo*-like PDAC model, also suggesting that the presence of stromal elements does not interfere with the anticancer potential of this novel combinatory approach.

Conclusions

Collectively, these results indicate that the combinatory approach between chemotherapeutic drugs and cell-based therapy is able not only to affect tumor cell viability but also induce stromal cell apoptosis in a PDAC *in vitro* model.