CHALLENGING PANCREATIC DUCTAL ADENOCARCINOMA & ITS STROMA BY A COMBINATION OF CHEMO & GENE THERAPY: A PRE-CLINICAL STUDY

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a gastrointestinal malignancy still characterized by unacceptable prognosis (*Malvezzi et al. 2014*). Treatments include surgery, chemotherapy, radiation therapy and palliative care and the most appropriate option is selected depending on the stage of PDAC (*Kamisawa et al., 2016; Vincent et al., 2014*). Among the most important factors contributing to the failure of treatments, a pivotal role is played by a pronounced desmoplastic stromal reaction composed by extracellular matrix (ECM), blood vessels, endothelial cells, connective tissue elements such as tumour associated fibroblasts (TAF), immune cells and soluble proteins such as cytokines and growth factors (*Erkan et al., 2012; Horimoto et al., 2012; Kalluri and Zeisberg, 2006*). Therefore, next to selective PDAC-targeting agents, approaches able to modify PDAC microenvironment may impact tumor/stroma interactions allowing a better therapeutic profile (*Bijlsma et al., 2015*). Since years, we have been developing a gene-therapy strategy based on adipose derived mesenchymal stromal cells (AD-MSC) to deliver the novel variant of the potent anticancer agent TRAIL (sTRAIL)(*Spano et al., 2019; Rossignoli et al. 2019*). Genetically modified AD-MSC delivering sTRAIL can reshape the PDAC microenvironment inducing tumor cell apoptosis (*Spano et al., 2019*). Starting from this concept, we here challenged the gene therapy approach in combination with standard anti-PDAC agents, such as Gemcitabine and Nab-Paclitaxel as pre-requisite for a phase I/II clinical trial.

Material and Methods

2D cytotoxicity assay. Target PDAC cells (28.000/well) were seeded in 12 well plates. Cells were pre-treated with Gemcitabine (GEM) for 24 h and then with sTRAIL for further 24 h. Tumor cell apoptosis was evaluated after 24h by FACS Aria III (Becton Dickinson) using propidium iodide (PI; 50 µg/ml; Sigma Aldrich). ***3D cytotoxicity assay.*** Luciferase positive tumor cells (560.000 cells) were loaded into the small bioreactor VITVO. Tumor cells were treated or not with chemotherapeutic drugs (10 uM GEM; 1 uM Nab-Paclitaxel) for 24 hours. Then, AD-MSC sTRAIL were delivered into the bioreactor at different E:T ratios (1:10 and 1:30). After 24, 48 or 72 h tumor cell viability has been quantified based on the BLI signal intensity. ***DR5 expression by FACS analysis.*** sTRAIL resistant BxPc-3, treated with 10 uM GEM and 1 uM Nab-Paclitaxel alone or in combination for 24h, were stained with APC-conjugated anti-TRAILR2/DR5 (Biolegend) and APC (Miltenyi Biotec Inc.) isotype controls. Analyses were performed with FACS. ***PDAC orthotopic mouse model.*** BxPC-3 LUC+ (1x106) were implanted in NOD/SCID mice (n=36) into the tail of pancreas by surgical procedure. Tumor engraftment was verified by in vivo BLI imaging at day 7 (IVIS LUMINA III, PerkinElmer). Mice were then separated in four study groups: control group (no treatment), GEM alone (50 mg/kg), GEM and AD-MSC sTRAIL (E:T 1:10), GEM and AD-MSC sTRAIL (E:T 1:30). Two doses of GEM (50 mg/kg) were administered intra peritoneally (i.p.) at one-week distance. AD-MSC sTRAIL, labelled with VIVO TRACK680 fluorescent agent (PerkinElmer), were administered intra-tumorally at day 33 by eco-guided injection (VEVO 2100, Visual Sonic, Fujifilm). Paraffin-embedded tumor sections were evaluated by haematoxylin-and-eosin staining (Sigma Aldrich).

Results

❖ Gemcitabine synergizes with AD-MSC s TRAIL to induce PDAC mortality in 2D and 3D in VITVO models

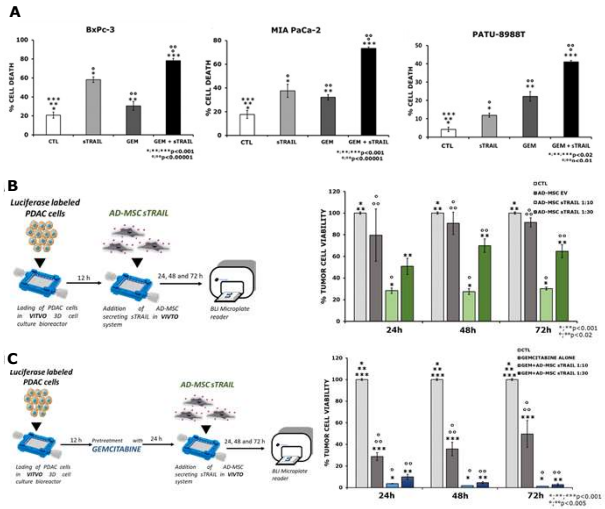

Figure 1. Gemcitabine cooperates with AD-MSC sTRAIL to induce PDAC death. 2D Dose Response assay revealed that combined treatment of GEM and sTRAIL provoked a significant increase in PDAC cell death rate compared to either sTRAIL alone or GEM alone (Figure 1A). A novel 3D bioreactor (**VITVO**), that allows to recreate a three-dimensional tumor in vitro, has been introduced to investigate the anticancer effect exerted by AD-MSC sTRAIL alone or in combination with GEM (Figure 1B;C). When used alone, the lower AD-MSC sTRAIL E:T ratio (1:10) was more effective in inducing tumor cell apoptosis and arresting tumor growth up to 72h compared to 1:30 (Figure 1B, right panel). In combination with GEM, both AD-MSC sTRAIL doses determined a massive cytotoxic impact higher than the one obtained by GEM alone confirming the synergy between GEM and AD-MSC sTRAIL in PDAC 3D model (Figure 3C, right panel).

❖ The combination GEM+AD-MSC sTRAIL provokes a massive destruction of the tumor parenchyma in orthotopic mouse model of human PDAC

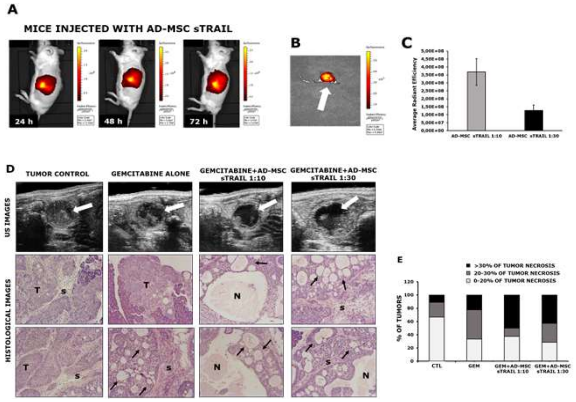

Figure 2. Persistence and impact of AD-MSC sTRAIL into PDAC tumor. Human PDAC orthotopic xenotransplant murine model has been developed to assess the AD-MSC sTRAIL persistence into PDAC tumor and to confirm the anti-cancer efficacy of the combination GEM+AD-MSC sTRAIL. AD-MSC sTRAIL were administered **intra-tumorally by eco-guided injection** and their biodistribution was assessed using infrared fluorescent labelling agent confirming their localization in the tumor area up to 7 days post-injection (Figure 2A-C). **Ultrasound (US) imaging system** revealed the presence of large necrotic tumor tissue, visible in the echographic images as intense hypoechoic (dark) regions, in treated tumors (Figure 2D, top row, white arrow). H&E staining confirmed that the hypoechoic regions correspond to large areas of necrotic (N) and empty spaces (black arrows) in tumor (T) parenchyma (Figure 2D, middle and bottom row). US analysis allowed to quantify the percentage of tumor necrosis revealing that in GEM+AD-MSC s TRAIL groups the majority of mice have higher levels of necrotic areas compared to GEM alone, with about half of animals with necrosis greater than 30% (Figure 2E). This indicated how addition of AD-MSC sTRAIL to GEM was able to significantly reinforce the anticancer effect due to the chemotherapeutic drug.

❖ Gemcitabine and Nab-Paclitaxel revert TRAIL resistance in PDAC cells

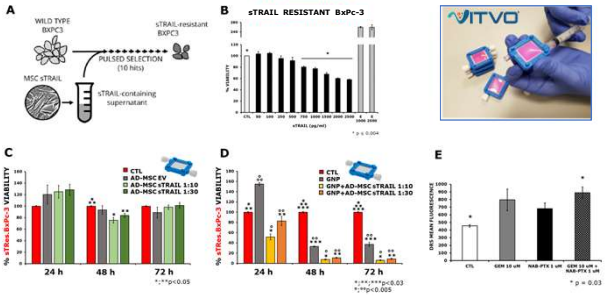

Figure 3. Gemcitabine + Nab-Paclitaxel treatment sensitizes resistant BXPC3 to sTRAIL. A sTRAIL resistant clones, obtained from parental BxPc-3 WT cells, has been established as represented in Figure 4A. sTRAIL resistant BxPc-3 (sTRes.BxPc-3) demonstrated 15-fold increase in sTRAIL tolerance compared to BxPc3 WT (Figure 4B). In 3D co-culture, AD-MSC sTRAIL at both 1:10 and 1:30 E:T ratios were not able to induce relevant cytotoxicity in sTRes.BxPc-3 (Figure 4C). However, when combined with Gemcitabine + Nab-Paclitaxel (GNP), AD-MSC sTRAIL showed a significant increase in tumor death rate compared chemotherapeutic drugs alone (Figure 4D). The combinatory approach with GNP significantly increase the expression of TRAIL surface receptor DR5 indicating one of the molecular mechanism underling the restoration of PDAC cells sensitivity to AD-MSC sTRAIL (Figure 4E).

Conclusions

Collectively, these results indicate the powerful potential of combining a gene therapy approach with chemotherapy suggesting the transferability of an anticancer MSC based approach in patients affected by PDAC

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