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Exosomes/EVs

Late Breaking Abstract

MSC-DERIVED EXTRACELLULAR VESICLES MODULATE METABOLIC ALTERATIONS IN HUMAN PULMONARY ENDOTHELIAL AND EPITHELIAL CELLS IN THE MODELS OF ARDS

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Keywords: Extracellular vesicles, Acute Respiratory Distress Syndrome, Metabolism.

Background & Aim: MSC-derived extracellular vesicles (EVs) are considered as a cell-free therapy for ARDS. Previously we demonstrated that MSC-EVs contain functional mitochondria and are able to restore activity of the injured cells through mitochondrial transfer. However, the impact of this mechanism on the balance of oxidative phosphorylation and glycolysis is not known. We hypothesized that therapeutic potential of MSC-EVs will depend on their ability to alleviate metabolic changes in primary human pulmonary cells exposed to LPS or ARDS plasma.

Methods, Results & Conclusion: EVs were isolated from BM-MSCs with normal or dysfunctional mitochondria by ultracentrifugation. Mitochondrial dysfunction in MSCs was induced by Rhodamine6G. EVs were characterized for number, size distribution, tetraspannin expression, and mitochondrial content. Primary human distal lung epithelial (HSAECs) and endothelial cells (HPMECs) were stimulated with LPS or plasma from ARDS patients and treated with EVs. Mitochondrial respiration and glycolytic flux were assessed by Seahorse metabolic analyser and barrier properties were assessed by xCELLigence. Relative contribution of mitochondrial respiration and glycolysis for the EV effect was assessed using selective inhibitors. Single cell transcriptomic analysis was performed on mouse lungs in the in vivoLPS-induced lung injury model. LPS or ARDS plasma stimulation resulted in pronounced reduction of mitochondrial respiration (35% and 28%), increase in glycolysis (30% and 33%) and functional impairment in HPMECs and HSAECs respectively. MSC-EVs isolated from normal MSCs inhibited glycolytic flux, restored mitochondrial respiration, and intracellular levels of ATP ($p<0.05$). MSC-EVs significantly restored barrier integrity in both cell types, inhibition of mitochondrial respiration abrogated this effect while inhibition of glycolysis did not. Treatment with mitochondria-depleted EVs was not effective ($p<0.05$). Analysis of the single cell sequencing data showed that MSC EVs administration regulates the expression of essential genes involved in mitochondrial metabolism in vivo. Stimulation with ARDS plasma impairs mitochondrial respiration and increases glycolytic flux in primary human SAECs and PMECs. Mitochondrial respiration is critical for barrier properties of both cell types while glycolysis is not. MSC EVs alleviate ARDS-induced metabolic alterations via transfer of healthy mitochondria. Funding: Horizon-2020-MSCA-IF ALGORITM 895134 to JS, MRC UK MR/R025096/1 and MR/S009426/1 to AK.

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Immunotherapy

TNFR2 AS A TARGET TO IMPROVE CD19-DIRECTED CART CELL FITNESS AND ANTITUMOR ACTIVITY IN LARGE B CELL LYMPHOMA

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Keywords: CART cell therapy, CRISPR/Cas9, Leukemia.

Background & Aim: Chimeric Antigen Receptor T (CART) cell activation and differentiation determine CART cell fate and response to therapy. Here, we aimed to measure levels of activation-induced death receptors and ligands on CART cells to improve their clinical responses.

Methods, Results & Conclusion: We performed flow cytometry on ex-vivo stimulated, clinically annotated CART products of patients from the ZUMA-1 clinical trial (Axi-Cel). We investigated possible correlations of surface death receptors and ligands with T cell differentiation status, post-infusion CAR T cell expansion, and response to therapy. CART cell effector functions and apoptosis were measured. For in vitro and in vivo functional studies, we used CART19 from healthy donors (HD CART19). First, we observed upregulation of death receptors and ligands in CART19 from non-responders, compared to responders after ex-vivo stimulation of Axi-Cel products. In an extended in vitro co-culture assay, where HD CART19 cells were repeatedly stimulated through the CAR, we found that tumor necrosis factor α receptor 2 (TNFR2), unlike other death receptors and ligands, was persistently elevated, suggesting a possible role for TNFR2 in long-term antigen-dependent CART19 dysfunction (Fig. 1A). We further found that HD CART19 upregulate TNFR2, but not TNFR1 upon CAR stimulation (Fig. 1B). While non-specific TCR activation of HD CART19 cells protected them from activation-induced apoptosis, antigen-specific activation through the CAR resulted in significant initiation of apoptosis within 2 hours of stimulation (Fig. 1C). Having identified a possible association between TNFR2 and CART19 dysfunction, we aimed to study the impact of TNFR2 knockout on HD CART19 functions. Using CRISPR/Cas9 during CART cell manufacturing, we generated $TNFR2^{k/o}$ HD CART19 cells (50% efficiency), where TNFR2 expression in activated CART19 cells was reduced, compared to control CART19 cells (Fig. 1D). $TNFR2^{k/o}$ CART19 cells demonstrated reduced early activation of CD25 and CD69 compared to control CART19 (Fig. 1E), reduced apoptosis initiation (Fig. 1F), and enhanced antigen-specific proliferation and cytotoxicity (Fig. 1G). Finally, in an in vivo xenograft model of $CD19^+$ lymphoma, $TNFR2^{k/o}$ CART19 resulted in enhanced CART cell expansion and antitumor activity (Fig. 1H). Our results indicate that TNFR2 plays a role in early activation and apoptosis initiation of CART19 following CAR stimulation with $CD19^+$ target cells and present TNFR2 KO as a strategy to enhance CART19 antitumor activity.