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31. PULMONARY VASCULAR HYPORESPONSIVENESS TO VASOCONSTRICTOR FACTORS IN HIGH-FAT DIET MICE. ROLE OF EXTRACELLULAR VESICLES

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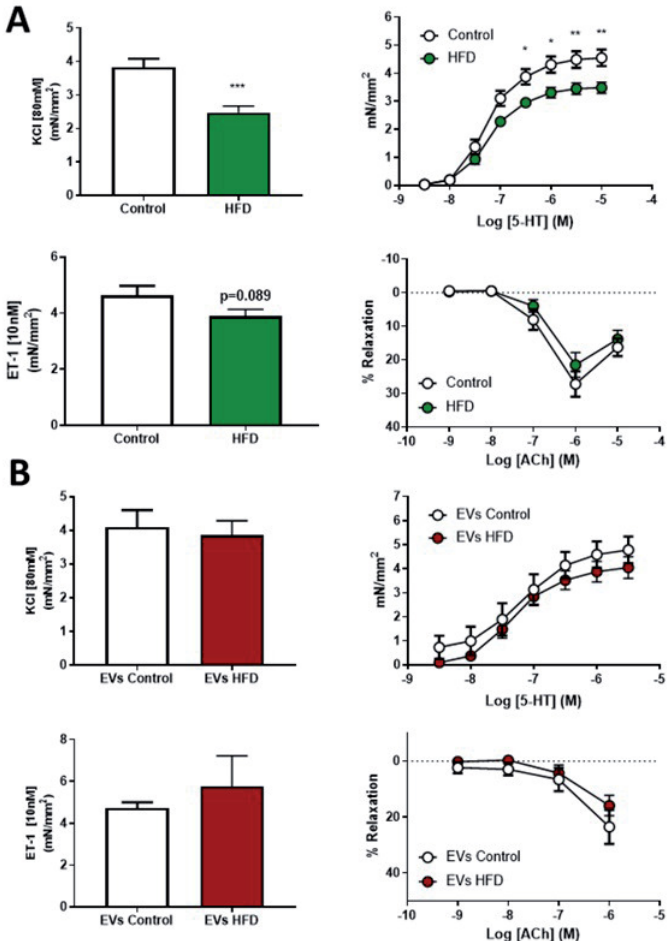
Introduction: Cirrhosis is the final stage of a series of chronic liver diseases, leading to high morbidity and mortality. Due to the growing epidemic of obesity and metabolic syndrome, non-alcoholic fatty liver disease (NAFLD) has become the most common cause of chronic liver disease worldwide, as well as a leading cause of cirrhosis. NAFLD is a risk factor for developing cardiovascular disease, type 2 diabetes and some lung diseases. Furthermore, liver cirrhosis can lead to two specific pulmonary vascular complications: Hepatopulmonary Syndrome (HPS) and Portopulmonary Hypertension (PoPH). There is increasing evidence that the release of extracellular vesicles (EVs) during liver cirrhosis could propagate damage to the lung and contribute to the development of pulmonary complications including PoPH and HPS.

Objectives: The main objective of the study was to investigate the involvement of hepatic EVs in the development of pulmonary vascular alterations in an animal model of high-fat diet-induced obesity.

Methods: 1. Animal model: female mT/mG mice (C57BL6/J genetic background) received high-fat diet (HFD, 45% fat for 12 weeks) to induce NAFLD *in vivo.* Livers were used to extract hepatocytes and to establish primary cultures. The left lung was used to assess pulmonary arteries (PAs) function by myography. 2. Myography: Freshly isolated conductance PAs were dissected, mounted, and exposed to different vasoactive factors. 3. Purification of EVs: EVs were obtained by differential ultracentrifugation using the conditioned medium obtained from in vitro hepatocyte cultures isolated from control and HFD mice. 4. Study of bioavailability of EVs in an in vitro model: control PAs were dissected and incubated in the presence of the hepatocytes-derived EVs for 20h at 37°C in DMEM medium. Vascular reactivity was then assessed.

Results: Treatment with HFD can affect the vascular reactivity of freshly isolated mice PAs. PAs of the HFD animals showed generalised hyporesponsiveness to vasoconstrictor factors (potassium, serotonin, endothelin-1) but not endothelium damage when measured as the ability of the vessels to relax to the endothelium-dependent vasodilator acetylcholine (ACh) (Figure A). However, treatment with hepatocytes-derived EVs did not induce any alterations in vascular reactivity (Figure B).



Conclusions: 1. Freshly isolated PAs from HFD mice exhibited hyporesponsiveness to potassium chloride, serotonin and endothelin-1, while maintaining preserved endothelial function. 2. Treatment with EVs secreted by HFD hepatocytes did not affect vasoconstriction or vasodilation function in PAs from control mice.
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36. IDENTIFICATION OF DIFFERENT LUNG IMMUNE-PHENOTYPES IN IDIOPATHIC PULMONARY FIBROSIS (IPF)

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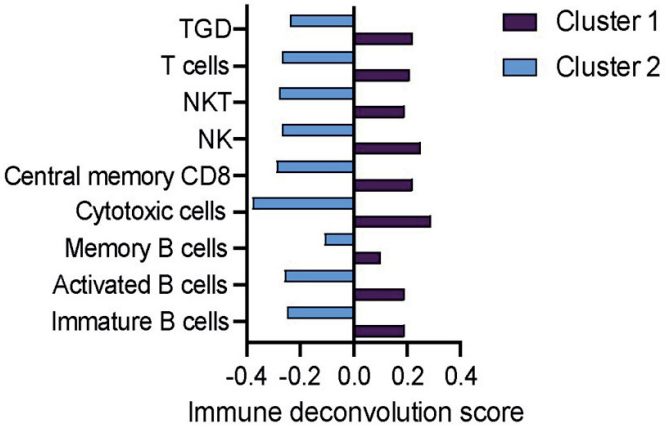
Introduction: Idiopathic Pulmonary Fibrosis (IPF) is a heterogeneous disease. The biological mechanisms underlying this heterogeneity are not well established. The putative role of the immune response in this setting is still controversial and traditionally has been largely neglected.
Objectives: To define the type and magnitude of the lung immune response in relation to specific pathological features.
Methods: To determine variability in the lung immune infiltrate, we applied transcriptomic deconvolution methods (Gene Set Variation Analysis (GSVA)) and unbiased clustering gene expression to IPF samples from the Lung Tissue Research Consortium (LTRC), n = 109. Main results were validated in two end stage IPF independent sets of samples: (1) fresh lung homogenates from the Pittsburgh University (n = 26), that were analyzed by flow cytometry, and; (2) lung tissue preserved in RNA-later and OCT slides from the CIBERES pulmonary biobank (n = 13) that were profiled by qPCR and immunohistochemistry techniques.
Results: Transcriptomic immune deconvolution of whole lung gene expression data from the LTRC identified two clusters differing in the overall level of immune infiltration (i.e. #1 immuno-infiltrated (55% patients) and #2 immuno-suppressed (45% patients). The most differentially expressed immune cell signatures corresponded to cytotoxic and B cells (Figure).These observations were reproduced in two sets of IPF samples, by measuring levels of T CD8 and B cells genes by qPCR and the proportion T CD3, B and NK cells by flow cytometry. Next, we explored differences in the gene expression between clusters and found 964 differentially expressed genes (with a FC > 1.5 and p-value < 0.05). We observed that the immune-depressed cluster was enriched in ciliated and secretory epithelial cells gene signatures (p-value of 2.68 10-10 and 1.28 10-6, respectively) and a key upregulated gene in the latter signature was MUC5B. Finally, in the CIBERES samples, preliminary results of MUC5B staining by immunohistochemistry confirmed higher MUC5B expression in the immune-depressed group. Interestingly, areas with higher epithelial remodeling and MUC5B expression did not co-localize with the immune infiltrate.



Conclusions: The type of lung immune infiltrate defines two groups of IPF patients, which are in turn associated with different lung pathological features. Weather these observations are associated with different prognosis remains unknown, however they open a new perspective on the potential role of the immune response as a main driver of IPF heterogeneity.
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