Transcriptomic and Metabolomic Analysis Reveals Pro-inflammatory and Pro-remodeling Phenotypes of Macrophages in Response to Adventitial Microenvironment in Pulmonary Hypertension

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Rationale

• The mechanisms driving macrophage polarization within the adventitial microenviroment during PH progression are still unclear.
• In pulmonary hypertension (PH), fibroblasts are the predominant cell type in the pulmonary artery where macrophages are most frequently observed. Persistently activated PH-Fibs produce cytokines, chemokines, growth factors, as well as the metabolites, and orchestrate a unique microenvironment in the PA adventitia to govern the phenotype and function of all cell types.
• We sought to determine the effects of the control and PH adventitial microenvironment on the transcriptomic and metabolomic profile of naive bone marrow-derived and identify the key pathways in control and disease.

Hypothesis

Macrophages undergo distinct transcriptomic and metabolomic programming in response to, and in coordination with, signals generated from fibroblasts in both control and pulmonary hypertensive perivascular microenvironments.

Methods

• Mouse bone marrow derived macrophage (BMDM) were treated with media conditioned by adventitial fibroblasts isolated from control (CO-CM) or pulmonary hypertensive (PH-CM) neonatal calves or left untreated (UNX). Samples were collected for RNA-seq and mass spectrometry analysis.
• Ingenuity Pathway Analysis (IPA) was used to analyze the RNA-seq data.
• The in vitro RNA-seq data of macrophages treated with adventitial fibroblast conditioned media was compared to the in vivo data of lung interstitial/perivascular macrophages flow sorted from mouse exposed to 4-day hypoxia.

Summary

1) PH-CM and CO-CM actively yet differentially regulate macrophage transcriptomic profiles: PH-CM activated pro-inflammatory, immune response, and metabolic canonical pathways in BMDMs, while CO-CM inhibited these pathways.
2) The upstream regulators identified in BMDMs treated with PH-CM or CO-CM are distinct and the differences lie in the cytokines, metabolites in the microenvironment, the receptors expressed on BMDMs, as well as activated or inhibited transcriptional regulators and signaling pathways.
3) PH-CM induced metabolic reprogramming in BMDMs with increased aerobic glycolysis, increased PPI, an altered TCA cycle, characterized by increased glutamine utilization, polyamine synthesis, and accumulation of amino acids, nucleotides, gamma-glutamylcys, and fatty acids in BMDMs compared to untreated and/or CO-CM treated BMDMs.
4) Canonical pathways regulating macrophage phenotype in vitro are closely related to the in vivo flow sorted lung interstitial/perivascular macrophages from hypoxic mouse.

Conclusion

Pulmonary adventitial microenvironment generated by fibroblasts regulate macrophage transcriptomic and metabolomic programs in pulmonary hypertension.