

Mechanisms and clinical implications of endothelial dysfunction in cystic fibrosis

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Background. Despite the involvement of endothelial cells (EC) in cystic fibrosis (CF) pathogenesis is suggested by (1) the emerging evidence of the risk for cardiovascular events in CF patients as well as by (2) our work showing endothelial dysfunction and its correlation with lung disease in CF patients, EC have not been extensively studied in CF.

Hypothesis and objectives. We hypothesized that the elucidation of CFTR signaling in EC may provide clues for innovative pharmacology for CF. Main objectives of this study were to: 1. Uncover mechanisms of CF endothelial dysfunction as molecular targets for novel therapeutics. 2. Elucidate the clinical relevance of circulating endothelial cells (CEC) and endothelial microvesicles (EMV) in CF.

Methods. Flow cytometry, confocal microscopy, western blotting, real-time PCR, electrophysiology.

Results. We isolated EC from pulmonary artery of explanted CF lungs (CF-PAEC) and measured CFTR activity using patch clamping. With experiments under static conditions or shear stress, we demonstrated that CF-PAEC displayed reduced monolayer integrity and trans-endothelial electric resistance (TEER), whereas they released a higher number of microvesicles (EMV). Similar results were obtained with PAEC and CF-PAEC immortalized with SV40 Large T antigen. CF-EMV had a different impact on PAEC and CF-PAEC proliferation and TEER compared to EMV, indicating that select transcellular

communication pathways via EMV are altered in CF. Along these lines, EMV and CEC were increased in peripheral blood of CF patients and inversely correlated with select respiratory indices. In an attempt to uncover molecular signatures of CFTR dysfunction in EC, we evaluated microRNA expression in PAEC and CF2-PAEC. CF2-PAEC, as well PAEC exposed to CFTRinh-172, expressed lower amounts of miR-216a-5p and 19a-3p, whereas they were enriched in miR-197-3p, 409-3p, 126-5p and 181b-5p. CFTRinh-172-treated PAEC also overexpressed (over two fold) miR-223-3p and 195-5p, whereas miR-376c-3p, 181a-5p, 216a-5p and 19b-3p were downregulated. Remarkably, agents that increase cAMP levels, such as type III and IV phosphodiesterase inhibitors and beta adrenergic receptor agonists, corrected TEER and monolayer integrity of CF-PAEC.

Spin-off for research & clinical purposes. Our results encourage further studies on miRNA and transcellular communication pathways as well as on the clinical utility of cAMP-increasing drugs to improve endothelial dysfunction in CF.

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