33 Linking protein N-glycosylation and CF: malfunctioning CFTR and pulmonary infection yield aberrant N-glycosylation of sputum derived proteins of CF individuals

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Objectives: Mucins are abundant glycoproteins in human lungs. It is well-established that airway mucin O-glycosylation is aberrant in cystic fibrosis (CF) and involved directly or indirectly in the pathogenesis. As the first, we here investigate and establish the link between protein N-glycosylation and CF.
Methods: N-glycosylation of crudely isolated sputum non-mucin proteins of five CF and five non-CF individuals with and without pulmonary infection was mapped using liquid chromatography and tandem mass spectrometry based glycomics and glycoproteomics. The resulting glycoprofiles were qualitatively and quantitatively compared between the patient groups.
Results: Despite covering different patient characteristics including CFTR genotypes, age, gender and microbial flora, the sputum N-glycomes showed little inter-person and longitudinal variation within the patient groups. Inter-group comparisons revealed that lung infection, primarily caused by *P. aeruginosa,* extensively altered the CF sputum N-glycosylation to paucimannoside rich profiles with simultaneous over-sialylation/fucosylation and under-bisecting GlcNAcylation of the complex N-glycans. The CF genotype in itself yielded fewer sputum N-glycome alterations by slightly increasing the abundance of paucimannose N-glycans in CF relative to pathogen-infected non-CF individuals.
Conclusion: We have established that the absence of a functional CFTR and more importantly the bacterial infection of the respiratory tract of CF patients affect their sputum N-glycosylation phenotype. This study provides an important platform to further understand the complex cellular and molecular environment of the respiratory tract in CF.

34 Alterations in cystic fibrosis peripheral blood mononuclear cells are induced by an increase in intracellular calcium concentration

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Introduction: We have previously observed that in peripheral blood mononuclear cells from CF patients (CF-PBMC) carrying the F508del CFTR, Ca^{2+} homeostasis is altered, promoting an abnormal activation of calpain, the protease of the Ca^{2+}-dependent proteolytic system. As a result, F508del-CFTR is cleaved by calpain, removed from the plasma membrane and internalized in endosomes.
Objectives: The involvement of calpain in the removal of F508del-CFTR from the plasma membrane of CF-PBMC is demonstrated by a large rescue of the chloride channel at the correct localization in cells exposed for 24–48 hours to a synthetic calpain inhibitor. Thus, the Ca^{2+}-dependent proteolysis participates, together with the proteasome activity of the ER-quality control mechanism, in producing the complete loss of CFTR at the plasma membrane. However, the alteration in Ca^{2+}-homeostasis could be further increased in PBMC of CF-patients following LPS stimulation during bacterial infections.
Methods and Results: In cells from all CF patients analyzed, large amounts of MMP9 are constitutively released into the extracellular medium. This process is completely prevented if the cells are preloaded with a Ca^{2+}-chelator. Exposure to LPS resulted ineffective, being the $[Ca^{2+}]_i$ already altered. PBMC from healthy donors are unable to constitutively release the MMP9 but this process is triggered following cells exposure to 50 μg/ml LPS.
Conclusion: Our observations are supporting the fact that PBMC from CF patients are functionally altered due to a persistent increase in $[Ca^{2+}]_i$, leading to activation of a number of Ca^{2+}-dependent processes, some of which acquire aberrant functions.