**SELDI-TOF-MS ProteinChip profiling of serum and nasal cells from Cystic Fibrosis patients**

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Surface enhanced laser desorption/ionisation-time of flight (SELDI-TOF) mass spectrometry (MS) is an array based proteomic technology. It combines a variety of chromatographic surfaces that bind proteins from biological mixtures according to physicochemical properties with MS analysis. The relative expression of proteins at specific molecular weights can then be compared among different samples. The aim of this work is to establish protein profiles in Cystic Fibrosis (CF) serum and airway cell lysate for biomarker identification. These proteins may serve as diagnostic/prognostic markers or even as new targets for CF therapy. Serum and nasal epithelial cells (collected by brushing) were obtained from CF patients (n = 13/15) and controls (n = 11/9). Protein extracts were applied to a range of ProteinChip surfaces (atomic exchange, cationic exchange and metal affinity) and analysed by SELDI-TOF-MS. The mass spectral data demonstrate a larger group of proteins with differential expression in serum compared to nasal cells lysate. 45 peaks (p < 0.05) were observed in control serum samples and in nasal cells lysate. Further work is required to validate these results in a larger cohort of CF patients and to identify candidate biomarkers with MS protein characterisation.

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**CFTR and MDR mRNA expression in patients with Cystic Fibrosis before and after 6 months of Azithromycin**

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Introduction: Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) and multidrug resistance (MDR1) are members of the "ATP-binding cassette" superfamily of transporters. Up-regulation of MDR has previously been shown after macrolide treatment in animals [1,2]. A study was performed to see if the same was true for patients with Cystic Fibrosis (CF).

Material and methods: 6 adult CF patients received AZM (500 mg daily) for 6 months. FVC% and FEV% and number of acute exacerbations were compared before and after treatment. Nasal biopsies were taken from the patients before and after AZM treatment and from age-and sex matched healthy controls. 45 cm2 cryosections were hybridized with oligonucleotide probes complementary to mRNA sequences encoding MDR1, MDR1b-MDR2, MDR1b-MDR1, CFTR-1, CFTR-2 and CFTR-3 as described previously [3].

Results: Significant improvement in FEV% and fewer exacerbations were observed among the patients. mRNA expression of MDR and CFTR was detected in all biopsies with no significant difference noticed after treatment.

Discussion: As shown by others, the alleviation of the CF patients' status after AZM treatment was not linked to MDR or CFTR up-regulation [4]. The anti-inflammatory effects previously described for AZM could be an explanation for the improvement seen among the patients [5].

References


**Basal control of CFTR gene expression by bicarbonate-sensitive soluble adenyl cyclase in Calu-3 cells**

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Cystic fibrosis is dominated by inflammation and the best-known modulators of CFTR gene expression are cytokines and transduction cytosolic pathways stimulated by inflammation. However, one may hypothesize that the expression of the CFTR gene, which encodes a well-defined anion channel, should also be controlled by CF or HCO3- concentrations. The effect of increasing extracellular HCO3- concentration was tested on the Calu-3 cell contents in CFTR mRNA and protein by Northern- and Western-blotting. Varying the HCO3- concentration of the basal culture medium between 0 and 50 mmol/l dose-dependently increased the CFTR expression, with an optimal effect observed with 25 mmol/l, and this resulted from enhanced CFTR gene transcription. No variation was observed on the expression of the cyclooxygenase-2 gene, chosen as an example of an inflammation-responsive gene, and the increase in CFTR mRNA cell content induced by the inflammatory cytokine IL-1β was not significantly modified by the extracellular bicarbonate concentration. The bicarbonate supplementation concomitantly induced a cAMP production inhibition by 2-OH E2, the selective inhibitor of the soluble adenyl cyclase, and intranuclear phosphorylation of the transcription factor CREB. Inhibition of this transduction pathway with either 2-OH E2 or the PKA inhibitor H-89 suppressed the bicarbonate-induced stimulation of basal CFTR gene expression.

Altogether, these results show that the CFTR gene expression is submitted to a double system of regulation corresponding to the double function of the protein involved in ion transport and response to inflammatory stimuli.

**Curcumin rescues deltaF508-CFTR via the keratin 18 network**

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The most common mutation in the CFTR gene, AF508, causes retention of AF508-CFTR in the endoplasmic reticulum and leads to the absence of CFTR Cl- channels in the plasma membrane. AF508-CFTR retains some Cl- channel activity so increased expression of AF508-CFTR in the plasma membrane can restore Cl- secretion deficiency. Recently, curcumin was shown to rescue AF508-CFTR localization and function. In our previous work, the keratin 18 network was implicated in AF508-CFTR trafficking. Here, we hypothesized that curcumin could restore a functional AF508-CFTR to the plasma membrane acting via the K18 network. First, we analyzed the effects of curcumin on the localization of AF508-CFTR in different cell lines (HeLa cells stably transfected with WT-CFTR or AF508-CFTR, CALU-3 or CFPAC-1 cells) and found it was significantly delocalized towards the plasma membrane in AF508-CFTR-expressing cells. We also performed a functional assay for the CFTR chloride channel in CFPAC-1 cells treated or not with curcumin and detected an increase in a cAMP-dependent chloride efflux in untreated AF508-CFTR-expressing cells. Then, the K18 network was analyzed by immunocytocchemistry and immunoblot exclusively in curcumin-treated or untreated CFPAC-1 cells because of their endogenic AF508-CFTR expression. After curcumin treatment, we observed a remodeling of the K18 network and a significant increase in K18 Ser52 phosphorylation, a signature of the reorganization of intermediate filaments. The last results will be presented.