81 Requirement of endosomal SNARE protein complex in CFTR activity/trafficking regulation

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We previously showed, using several cell types, that the endosomal SNARE protein complex (described to mediate fusion of endosomes) is involved in CFTR regulation. Notably, we evidenced by co-IP experiments that CFTR could interact with each of the four endosomal SNAREs (syn7, syn8, vti1b and VAMP8). Moreover, overexpression of any of these proteins (or siRNA-induced vti1b depletion) elicited a dramatic decrease of CFTR channel activity, as monitored by iodide efflux assay. This was correlated to the loss of plasma membrane localization of CFTR which was then only found in perinuclear vesicles decorated by Rab11a protein, namely the recycling endosomes.

To understand how the SNARE proteins are involved in CFTR regulation we are currently testing in which transport step the correct endosomal SNARE complex activity is required. Using HeLa cell lines stably expressing WT- or F508del-form of CFTR, we established that RNA interference of any of the four SNAREs reduces WT-CFTR activity (this also seems to be the case in 16HBE14o- cells endogenously expressing WT-CFTR), without affecting the B to C band maturation efficiency. Interestingly SNARE depletions were without effect upon temperature-rescued F508del-CFTR activity in HeLa cells. Finally, using cell surface biotinylation experiments, the involvement of SNARE proteins on CFTR plasma membrane targeting, internalization and recycling is presently tested.

Since F508del-CFTR ER-export can be rescued in several ways without fully correcting post-ER defects, our work will supply new informations on the mechanisms involved in CFTR regulation after plasma membrane targeting.

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82 Increased interaction of F508del-CFTR with K8/K18 cytokeratin network by direct binding of K8 to NBD1

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We have previously reported an increased expression of cytokeratins 8/18 (K8/K18) in cells expressing F508del-CFTR. This is associated with colocalization of CFTR and K18 in the vicinity of the endoplasmic reticulum, while this is reversed by curcumin, resulting in the rescue of F508del-CFTR. We aimed to investigate if the K8/K18 network interacts directly with CFTR and, if confirmed, to precise the interaction site. Firstly, CFTR was immunoprecipitated from HeLa cells transfected with either wild-type CFTR, F508del-CFTR, or pTracer alone. Precipitates were subjected to 2D-electrophoresis and differential spots identified by MALDI-TOF. K8 and K18 were significantly increased in F508del-CFTR precipitates. In a second approach, by surface plasmon resonance, we immobilized mouse NBD1, either normal or bearing F508del, and used K8 and K18 as analytes. K8 strongly interacted with both NBD1 forms, while K18 binding was limited. The affinity constants of K8 were 42 nM for normal and 27 nM for mutated NBD1. The affinity for human NBD1 was the same as for the mouse molecule. Binding of K8 alone was stronger than in the presence of K18. In all cases, the interaction was totally inhibited by 10mM ATP. These results confirm a physical interaction of CFTR with the cytokeratin network and suggest a major contribution of K8 binding to NBD1. As F508del mutation primes binding affinity, this interaction may play a role in the defective trafficking of F508del. This suggests the cytokeratin network as a potential therapeutic target in CF.

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83 The calpain-caspase 12-caspase 3 cascade leading to apoptosis is altered in F508del-CFTR expressing cells

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In cystic fibrosis (CF) the most common mutation of the cystic fibrosis transmembrane conductance regulator (CFTR) is a missing phenylalanine at position 508 (F508del). The F508del-protein is incorrectly folded and retained in the endoplasmic reticulum (ER). Eukaryotic cells respond to the accumulation of misfolded proteins in the ER by activating the unfolded protein response (UPR). We previously showed that the UPR may be triggered in CF. Since prolonged UPR activation leads to apoptosis via the calcium (Ca^{2+})-calpain (Cal-1)-caspase 12 (Csp-12)-caspase 3 (Csp-3) cascade and because apoptosis is altered in CF, our aim was to compare the ER stress-induced apoptosis pathway between wild type (Wt) and F508del-CFTR expressing cells. The comparison was also studied after UPR induction by thapsigargin (Tg). We first assessed the cell viability of Wt and CF cells and observed a decreased cell death of the CF cells. Then we compared the intracytosolic Ca^{2+} concentration ($[Ca^{2+}]i$) between Wt and CF cells by microfluorometry and showed a lower increase of $[Ca^{2+}]i$ in CF cells after Tg treatment. Using western blot experiment we studied Cal-1, Csp-12 and Csp-3 expression in Wt and CF cells without Tg treatment and showed a decreased basal expression of these proteins in CF cells. Moreover we observed a lower and delayed increase of Cal-1 and Csp-12 expression and especially no accumulation of the active form of Csp-3 after Tg treatment in CF cells. Finally we studied Csp-12 and Csp-3 activities in Wt and CF cells by fluorometry and showed a decreased and delayed Csp-12 activation and a Csp-3 activation defect in CF cells. In conclusion we show that the altered apoptosis observed in CF under stress conditions (inflammation, infection) involves an altered Cal-1, Csp-12 and mostly Csp-3 activation

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84 Pharmacological inhibition of CFTR modifies cyclooxygenase-1 and -2 expression and PGD2 production in Calu-3 cells

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Pharmacological inhibition of CFTR with the specific inhibitor CFTRinh172 has been shown to induce a Cystic Fibrosis phenotype with enhanced NFkappaB activation and IL-8 secretion. Altered prostaglandin production has also been observed in Cystic Fibrosis, but its origin and characteristics remain unclear. The present work was undertaken to-determine whether the CFTRinh172 was also able to alter the pattern of the cyclooxygenase-1 and -2 expression and the production of PGD2 in Calu-3, the human pulmonary cells with a very high expression of an active CFTR.

By Western blotting, we found that treating the cells for 48 hours with CFTRinh172 (10 microg/ml)increased by 15 and 30% respectively the amounts of COX-1 and COX-2 proteins present in the internal membranes of the control cells (confluent cells maintained without FCS). The CFTRinh172 was no longer active on the COXs expression when Il-1beta (which increases COX-1 expression by about 20% and practically doubles that of COX-2) was administered during the 3 last hours of the incubation. The PGD2 content of the same cells was measured by immunoenzymatic assay. Inhibiting CFTR during 48 hours decreased their PGD2 content by about 30%. The PGD content of the control cells was also decreased by 35.5% after a 3hour-incubation with IL-1beta, but, on the opposite, was practically doubled in the cells treated with CFTRinh172.

These preliminary data suggest the existence of a complex relationship between CFTR activity, cyclooxygenases expression, and PGD2 production.