Assembly of MUC2 N-terminal with relevance for mucus formation

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MUC2, the main gel forming mucin in intestine, is stored as a densely packed multimter in goblet cell secretory granules at high calcium and low pH conditions. This mucin is in contrast to normal small intestine attached to the epithelium in Cystic Fibrosis, something that is likely linked to impaired release and expansion of the packed multimter. The MUC2 N-terminal part contains the von Willebrand D1D2D3 domains. These domains govern packing of the MUC2 polymer into a concatenated polygon platform by pH- and calcium-dependent non-covalent interactions of the D1D2 domains and disulfide-bonded covalent trimerization of the D3 domain. Following secretion the branched, net-like, MUC2 polymer is organized as stratified sheets. To obtain further insights into the structure of the MUC2 N-terminal trimer, the D3 domain, containing structural information promoting packing and release, was expressed, purified, and analyzed by subsequent gel filtration, transmission electron microscopy and single particle image processing. Sequence comparison to the von Willebrand factor (VWF) revealed that the D3 domain of MUC2 had a similar subdomain organization, namely, VWD, C8, trypsin-inhibitor-like (TIL) and E in the order, TIL-E-VWD1-C8-3-TIL-E-3. The expressed protein was a covalent trimer that upon gel filtration eluted as a hexamer. The obtained 3D maps, revealed a hollow, cage-like structure where six monomers were arranged as a dimer of trimers, confirming that the MUC2 mucin forms branched and not linear structures. Harriet E. Nilsson and Daniel Ambort have contributed equally.

WS12.2 Mucins are abnormally concentrated in CF respiratory secretions: role in disease pathogenesis

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The pathogenesis of cystic fibrosis lung disease remains unclear. The “2-gel” description of mucus clearance predicts that mucus flow depends on mucin concentration and high concentrations of secreted mucins produce muco-obstructive lung disease. We therefore reiterated the mucin concentration in CF sputum using immunological and biophysical techniques. Mucin concentrations were lower in CF sputum when measured via immunological techniques. However, mass spectroscopic analyses of mucin revealed substantial cleavage of the exposed regions of CF mucins at antibody recognition sites. Accordingly, total mucin concentrations in CF secretions measured by HPLC/refractometry were ~3× higher than normal. Parallel Multi Angle Light Scattering studies of mucin oligomeric structures revealed that normal mucins that were treated with neutrophil elastase and mucins isolated from CF remained intact. A novel technology developed to measure the partial mucin osmotic pressure (π) of respiratory samples revealed that CF sputum π was ~5× higher than normal sputum and the immobile mucins in CF lungs exhibited an ~10× higher π. We conclude that mucin concentrations cannot be accurately measured immunologically in the proteolytic environment of CF secretions, macromolecular integrity of the mucins is not effected from the proteolytic cleavages to some degree, mucins are hyperconcentrated in CF secretions measured biophysically, and the osmotic pressure measurements are consistent with the hypothesis that there is osmotic compression of the PCL by the mucus layer and the resultant mucus stasis and mucus stasis-driven infectious and inflammatory components of CF pathogenesis.

WS12.3 Detachment of mucus requires a specific proteolytic cleavage in the MUC2 mucin explaining why the cystic fibrosis mucus is attached to the epithelium

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Objectives: The mucus of the intestine is a highly organized system which protects the epithelia against microbial challenges from the outside. The mucus layers consist of one major building brick, the MUC2 mucin, which is produced and secreted by goblet cells. This extensively glycosylated molecule forms a loosely structured layer in the ileum which is normally not attached to the epithelium. In contrast, we found recently, that the ileal mucus of Cystic Fibrosis (CF) mice is attached. Here, we describe a mechanism which can explain the attachment of the mucus in this disease.

Methods: Using an Ussing-type explant system, the attachment of the mucus in the small intestine of CF mice could be reversed by the addition of bicarbonate. Bicarbonate is suggested to be necessary for the normal unfolding of the densely packed mucin.

Results: We have now discovered that the zinc-dependent metalloprotease meprin β is responsible for the detachment of the mucins from the epithelium by a cleavage in the MUC2 mucin. Accordingly, the mucus of meprin β-deficient mice was attached and could be released upon addition of the recombinant enzyme. The treatment of CF mucus with the recombinant meprin β alone had no effect. However, the addition of bicarbonate released the CF mucus from its attachment.

Conclusions: The findings suggest that unfolding is necessary for exposing the meprin β cleavage sites for releasing the attached CF mucus. Consequently, the unfolding process is impaired in CF. This novel mechanism enables us to shed some light on basic processes in Cystic Fibrosis.

WS12.4 Planar cell polarity protein network, which controls ciliogenesis and cilia function, is altered in human cystic fibrosis bronchial epithelial cells through response to endoplasmic reticulum stress

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Objectives: Microrcellular clearance (MCC), abnormal in CF lung, is regulated by several mechanisms including epithelial cilia movement. Planar Cell Polarity (PCP) has been described in mammal lung epithelial cells as a crucial mechanism controlling ciliogenesis and cilia function. Although the majority of studies on cilia in CF showed no structural abnormality and normal cilia beat frequency, cilia disorientation was showed to occur secondary to lung inflammation. We hypothesized that CF HBEs display abnormal PCP network and that could further impair coordinated cilia function.

Methods: We quantified expression of several PCP genes in HBEs and observed influence of CFTR genotype on PCP gene expression. By Western-Blot and immunofluorescence, we determined which components of PCP network were controlling ciliogenesis and cilia function. Although the majority of studies on cilia in CF showed no structural abnormality and normal cilia beat frequency, cilia disorientation was showed to occur secondary to lung inflammation. We hypothesized that CF HBEs display abnormal PCP network and that could further impair coordinated cilia function.

Results: We demonstrated that HBEs expressed several PCP genes. Among them, expression of CELSR3 (Cadherin EGF LAG seven-pass G-type receptor) was down-regulated in CF (F508del-CFTR rescue, ER stress inducing) to determine which causes PCP dysfunction in CF-HBEs. CELSR3 is a major building block of the PCL that is responsible for the attachment of the CF mucin explaining why the cystic fibrosis mucus is attached to the epithelium.