Cystic Fibrosis liver disease: role of CFTR as regulator of epithelial innate immunity

Introduction

Cystic Fibrosis (CF) is a common autosomal recessive disorder, affecting 1 in every 3000 live birth. CF is caused by mutations in CFTR, a protein that regulates fluid secretion in a number of organs. In CF patients the disease can be complicated by liver disease (CFLD), a condition that can compromise survival and quality of life of these patients. Unfortunately a cure is not yet available. Defective CFTR function impairs the ability of specialized liver cells to produce bile in the proper amount and quality, however we have recently described that lack of CFTR has a profound impact on the defense mechanisms (TLR signaling) that normally protect the biliary system from infections (innate immunity). Hypothesis and aims: Our hypothesis is that CFTR participates to the regulation of TLRs signaling and that a correct therapeutic approach should aim at controlling inflammation in biliary epithelial cells. Therefore we studied the molecular mechanisms linking CFTR with TLR signaling and we validate the potential therapeutic value of PPAR-γ agonists to treat inflammation. Spin-off for research & clinical purposes. These studies unveil novel mechanisms of epithelial physiology and innate immunity. Our findings suggest that altered innate immune mechanisms of the biliary epithelium are responsible for chronic inflammation, typical feature of CFLD, and that a correct therapeutic approach should be target the TLR-driven inflammatory response. Future plans: In the future, we are planning to use human induced pluripotent stem cells (iPSCs) derived from CF patients with different mutations to differentiate human biliary cells and model the human disease to test the therapeutic approaches targeted in the mouse model.

Project 1

Role of PPAR-γ in the modulation of inflammation in cystic fibrosis biliary epithelium

Background and Aims of the project: Cystic Fibrosis-associated liver disease (CFLD) is a chronic cholangiopathy that negatively impacts the quality of life and survival of CF patients. Our recent studies show that in CFTR-defective cholangiocytes, TLR-4/5-dependent innate immune responses are increased and may contribute to the pathogenesis of CFLD. Our studies imply that a correct therapeutic approach to CFLD should aim at controlling inflammation in biliary epithelial cells. Emerging evidence support a role of the nuclear receptor (NR) PPAR-γ as negative regulator of TLR-mediated inflammation. In this study, we tested the hypothesis that pharmacological activation of PPAR-γ would limit the altered innate immune response in CFTR-defective biliary epithelium.

Results: 1. Protein expression of nuclear receptor PPAR-γ is increased in CFTR-defective cholangiocytes.

Western blot of PPARγ in cystic and nuclear protein fractions from WT and Chf-KO (CF) cholangiocytes. Data were performed in n=3 experiments. *p<0.05 vs WT.

2. CFTR-KO cholangiocytes show an imbalance between ω-3 and ω-6 polyunsaturated fatty acids (PUFAs).

Lipidomic analysis of total lipids extraction from WT and CFTR-KO (CF) cholangiocytes. A: arachidonic acid, DHA, docosahexaenoic acid LA: linoleic acid. Data were performed in n=4 experiments. *p<0.01.

3. In vivo PPAR-γ activation reduces biliary damage and inflammation in Chf-KO mice treated with DSS

Chf-KO mice treated with DSS or with DSS and rosiglitazone. At the end of the treatment, liver tissues were stained with the cholangiocyte-specific marker K19 (A) or the leucocyte specific marker CD45 (B). Computer-assisted morphometric analysis of K19 and CD45 positive areas were performed. Rosiglitazone treatment significantly reduced the bile duct proliferation and inflammatory cell infiltration in Chf-KO mice treated with DSS. *p<0.05 vs DSS only.

Conclusions/Perspectives (P1)

- Stimulation of Hils may represent a novel strategy to control inflammation in CF biliary epithilum.
- Mechanisms described in CF liver disease may potentially be extended to other chronic inflammatory liver diseases.

Project 2

CFTR-dependent regulation of innate immunity and epithelial polarity in cholangiocytes through c-Src tyrosine kinase signaling and regulates

Background and Aims of the project: CFTR is expressed at the apical membrane of cholangiocytes where it regulates Cl- and HCO3- secretion. CFTR also modulates innate immune responses in the biliary epithelium. In fact, TLR4-mediated responses to LPS are increased in cholangiocytes from Chf-KO mice along with the activity of c-Src, a non-receptor tyrosine kinase. Aim of this study, was to understand how CFTR deficiency leads to up-regulation of c-Src activity in cholangiocytes.

Results: 1. CFTR interacts with proteins regulating 6-c activity

A. Lysates from WT and Chf-KO cells were immunoprecipitated with antibodies against c-Src or Cbp and immunoblotted with specific antibodies against CFTR, Cbp and EBP50. The Western blot analysis shows that CFTR, Cbp and EBP50 are physically interacting in WT cells but not in Chf-KO cells. B. Confocal images of polarized WT and Chf-KO cells stained for EBP50 or for CFTR+Cbp-Caik or TLR4-Caik. EBP50 is specifically localized on the apical membrane in WT cells while appears diffused and overlaminated in Chf-KO cells. 2. Conclusions: show that CFTR, Cbp and Cak co-localize on the apical membrane in WT cells, but not in Chf-KO cells. TLR4 shows the same distribution in WT and CF cells, but do not co-localize with Cak in CF cells.

2. In vivo inhibition of Src decreases biliary damage and inflammation in Chf-KO mice treated with DSS

Chf-KO mice were untreated or treated with DSS or with DSS and PP2A. At the end of the treatment, liver tissues were stained with the cholangiocyte-specific marker K19 or the leucocyte specific marker CD45. Computer-assisted morphometric analysis of K19 and CD45 positive areas were performed. PP2A treatment significantly reduced the bile duct proliferation and inflammatory cell infiltration in Chf-KO mice treated with DSS (*p<0.05, **p<0.01).

Conclusions/Perspectives (P2)

- A) In the normal epithelium, CFTR, Src and TLR4 are localized in the apical membrane. Cak translocates to the membrane to phosphorylate and maintain Src in an inactive state. Cak is a cytoplasmic protein and requires the membrane adaptor Cax Binding Protein (CBP), to localize in close proximity and inhibit Src. CBP is anchored to the cytoskeleton by PDZ domains of EBP50, known to be also associated with CFTR. B) In the absence of CFTR, the consequent destabilization of EBP50 prevents the stabilization of CBP, the translocation of Cak and consequently Src changes to an active state. Activated Src phosphorylates TLR4 increasing its response to LPS, destabilizes the structure of cell-cell junctions and alters the epithelial barrier function, therefore facilitating the passage of toxic bile acids that further damage the epithelium.

- B) Cytokines

- Cytokines