Ruprecht-Karls-Universität Heidelberg
Medizinische Fakultät Mannheim
Dissertations-Kurzfassung

FXR and FOXA2 govern two complementary regulatory mechanisms to maintain apical BSEP expression in hepatocytes

Autor: Sai Wang
Institut / Klinik: II. Medizinische Klinik
Doktorvater: Prof. Dr. S. Dooley

Background & Aims: Under a favorable microenvironment, nuclear receptor FXR controls apical ABC-transporter BSEP/ABCB11 expression for bile acid delivery. In acute-on-chronic liver failure (ACLF), most patients lack nuclear FXR in hepatocytes, but frequently maintain apical BSEP expression. The current study investigated the mechanisms how hepatocytes are able to maintain BSEP expression in the absence of FXR.

Methods: We investigated liver tissues from 3 healthy controls, 5 chronic HBV infected, and 18 ACLF patients. Among ACLF patients, 13 received liver transplantation, whereas 5 recovered. BSEP and target transcription factors were examined by immunohistochemistry in the collected liver tissues. BSEP expression regulatory mechanisms were investigated in AML12 cell line, mouse primary hepatocytes, human primary hepatocytes and *fxr* knockout mice.

Results: Firstly, immunohistochemical staining (IHC) in 26 liver tissues showed intact apical BSEP expression in control samples, chronic HBV infected and recovered ACLF patients. In the cohort of irreversible ACLF patients, 10 were positive for BSEP staining in most hepatocytes, while 3 have lost BSEP expression in most areas of the specimen. Fifteen ACLF patients with maintained BSEP expression did not have detectable nuclear FXR expression. Instead, they robustly expressed FOXA2 in the nuclei of hepatocytes. We compared clinical parameters between ACLF patients possessing and lacking BSEP expression. Recovered patients show remarkably improved serum total bilirubin, international normalized ratio and Model for End-stage Liver Disease scores than those in need of receiving liver transplantation (p<0.01 for all parameters). In addition, compared to the irreversible patients with BSEP, patients who lost apical BSEP presented with higher serum total bilirubin concentrations (p<0.05). These results suggest that maintenance of BSEP in hepatocytes is important for the recovery from ACLF.

In vitro, overexpression or knockdown of FOXA2 induced or inhibited BSEP expression in hepatocytes. Furthermore, ectopic FOXA2 expression restored apical BSEP expression in hepatocytes of *fxr* knockout mice *in vitro* and *in vivo*. ChIP assays revealed that both, FXR and FOXA2, initiated *ABCB11/BSEP* transcription through binding to the gene promoter. However, administration of an FXR agonist reduced FOXA2 binding to the *ABCB11/BSEP* gene promoter, indicating a competitive effect between FOXA2 and FXR on binding to the *ABCB11/bsep* gene promoter. In normal hepatocytes, insulin impeded FOXA2 nuclear translocation, while glucagon induced FOXA2 expression. Under inflammatory conditions, as present in ACLF, inflammatory factors, e.g. LPS and TNFα, induce high levels of glucagon and inhibit FXR expression. Further, TNFα induces hepatocyte insulin resistance and thus initiates FOXA2-dependent BSEP expression. We further found that LPS initiated *foxa2* transcription via NFκB signaling and p65 binding to the *foxa2* promoter. Notably, high concentrations of LPS led to FOXA2 nuclear exclusion through phosphorylation at Thr156, which subsequently led to reduced apical BSEP expression in hepatocytes.

Conclusions: The findings of this study illustrate that two regulatory mechanisms are relevant for the maintenance of BSEP on bile canaliculi in physiological and pathological conditions.

In physiological conditions, FXR regulates BSEP expression.

In inflammatory circumstances, FXR function is significantly compromised. Inflammation-dependent insulin resistance, high levels of glucagon and NFκB p65 signaling induce FOXA2 expression and activity to maintain BSEP expression, which is important for ACLF patient survival and recovery.

In sepsis conditions, severe infection and inflammation, e.g. high levels of LPS, inhibit FOXA2 activation and thus lead to loss of BSEP expression on bile canaliculi, which is associated with poor prognosis of ACLF patients.

In conclusion, maintenance of BSEP is essential for survival and recovery of ALCF patients.