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Targeting bile salt-FGF19 signaling

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Appendix I

Summary

Bile salts not only aid in absorption of dietary lipids, but also act as signaling molecules controlling negative feedback regulation of synthesis of bile salts, lipogenesis, and gluconeogenesis in the liver. The bile salt-fibroblast growth factor 19 (FGF19) signaling axis depends on an intact enterohepatic circulation (EHC) of bile salts, and is important for gut-liver health including promoting liver regeneration and improving intestinal failure (IF) and intestinal failure-associated liver disease (IFALD).

In **Chapter 1** we provided a general introduction to this thesis. We first introduced the physiology of the EHC of bile salts, the impact of biliary obstruction (e.g. perihilar cholangiocarcinoma, pCCA) on the EHC, and the interventional strategies (i.e. preoperative biliary drainage, BD) to decompress the biliary obstruction. Furthermore, we discussed the interrupted EHC due to loss of the 'source' of bile salts, such as partial hepatectomy, and the potential role of bile salt signaling on portal vein embolization (PVE)-induced liver hypertrophy. Moreover, we introduced disturbance of the EHC because of outflow of succus entericus in the stoma bag in patients with a temporary jejunal double enterostomy, and beneficial effects of chyme reinfusion (CR) in improving IF and IFALD. Finally, the aim and outline of this thesis were summarized.

In **Chapter 2** we gave a systematic review of systematic reviews to assess the effect and route of preoperative BD in patients with resectable perihilar cholangiocarcinoma (pCCA). Through searching database, eleven systematic reviews (SRs) with meta-analysis including 5950 patients were identified. All but one original studies in the SRs were retrospective. Ten of eleven SRs had high risk of bias. For preoperative BD *versus* no preoperative BD, all SRs showed no statistical differences in postoperative mortality. Preoperative BD was associated with increased postoperative major morbidity in 'simple criteria' patients receiving BD only based on the presence of jaundice. For endoscopic biliary drainage (EBD) *versus* percutaneous transhepatic biliary drainage (PTBD), three of four SRs showed that the postoperative mortality was not significantly different between the two drainage modes. EBD was associated with higher drainage-related overall morbidity, cholangitis and pancreatitis rates in three of four, three of five, and four of four SRs, respectively. PTBD was associated with higher seeding metastasis rates and worse postoperative overall survival. This study does not end the debate on the significance and preferred route of preoperative BD. The preoperative BD might need to be performed in strictly selected patients in terms of cholangitis, bilirubin levels and future liver remnant volume to avoid increased postoperative major morbidity. EBD might be associated with higher short-term

drainage-related morbidity but more favorable long-term oncological outcomes.

In **Chapter 3** we explored the suitability of human precision-cut liver slices (hPCLS) as an *in vitro* model to study human liver regeneration. hPCLS were derived from tumor-distal liver tissue from patients who underwent partial liver resection for colorectal liver metastases (CRLM). hPCLS were incubated with lipopolysaccharide (LPS), recombinant FGF19, and nuclear bile salt receptor agonists (obeticholic acid [OCA], farnesoid X receptor [FXR] agonist; 6-(4-Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde O-(3,4-dichlorobenzyl)oxime [CITCO], constitutive androstane receptor [CAR] agonist; rifampicin, pregnane X receptor [PXR] agonist). 5C and Geltrex were used to assess their effectiveness in maintaining expression of genes related to a differentiated hepatocyte phenotype. mRNA expression of IL6, FXR/FGF19 pathway-related genes and proliferation-related genes were assessed. We found that FGF19 did not induce *IL6* mRNA expression in hPCLS. In addition, marked phosphorylation of extracellular signal-related kinase (ERK)1/2 was already observed in untreated liver slices. FGF19 had no additional effect on levels of phosphorylated ERK1/2. Expression of FGF19 pathway-related genes (*CYP7A1*, *FGFR4*, *KLB*, *PCK1*) rapidly declined upon hPCLS culturing. The use of 5C and Geltrex did not maintain expression of these genes during culturing of hPCLS. Furthermore, nuclear receptors agonists OCA and rifampicin did not induce cell proliferation in hPCLS. The efficacy of FGF19 and CAR treatment could not be ascertained in these exploratory experiments. Hence, questions regarding the involvement of the FGF19/IL6 axis in hepatocellular proliferation, and augmentation of liver growth by CAR agonism could not be answered with the current method of hPCLS source, preparation and culturing.

In **Chapter 4** we investigated the mechanisms by which obeticholic acid (OCA) accelerates liver hypertrophy following PVE in a rabbit model. Specimen were analyzed from a previous experiment in which rabbits were treated by oral gavage with OCA (10 mg/kg, n=11) or vehicle (control group, n=11) prior to, and during the course of, PVE. Serum total bile salts (TBS), bile salt composition, C4 (marker of bile salt synthesis), and liver synthetic function were determined before, after 3 hrs, and at day 1, day 3, and day 7 after PVE. Ileal and hepatic genes expression were analyzed through targeted and untargeted approaches at day three after PVE, when the growth-enhancing effect of OCA was maximal. For correlation analyses, volumes of (non)embolized liver segments, and %volume gain, were previously determined by CT scan. We previously reported that PVE induced a larger volume gain of the non-

embolized liver lobe in OCA-treated animals. Effects of OCA were largest at 3 days after PVE (volume gain: 56.1% vs. 26.1% in OCA- and vehicle-treated animals, respectively). OCA treatment led to earlier normalization of serum TBS levels and lower bile salt content of the non-embolized liver lobe after PVE. OCA decreased mRNA expression of *Cyp7a1* (involved in bile salt synthesis) and promoted expression of *Slc51a/b* (involved in bile salt export) in the non-embolized liver lobe, likely contributing to the observed improved serum and liver bile salt homeostasis. Levels of lithoholic acid (LCA), a hepatotoxic bile salt, were lower in the OCA-treated animals compared to the control animals at day 1 and day 3 (P=0.002 and P=0.031, respectively). LCA levels were negatively related with caudal (i.e. non-embolized) liver volume (CLV) increase and the numbers of Ki-67 positive hepatocytes in this lobe (ρ = -0.726, P< 0.001; ρ = -0.718, P= 0.009, respectively). Both targeted and untargeted transcript analysis revealed associations with genes engaged in bile salt homeostasis and liver growth, adding to the notion that OCA-mediated improvement of bile salt homeostasis was in part responsible for enhanced PVE-induced liver growth. Additional factors candidates for mediating growth-stimulating effects of OCA include the secreted factors Bmp3 and Ihh. Our findings indicate that OCA improved bile salt homeostasis, which partly contributed to augmented hypertrophy of the non-embolized liver lobe in rabbits. OCA may be promising to increase the efficacy of PVE in patients with small future liver remnant planned for (extended) partial liver resection.

In **Chapter 5** we assessed the influence of cholestasis on PVE-induced future liver remnant (FLR) hypertrophy. Patients were enrolled with pCCA or CRLM, who underwent PVE before a (extended) right hemihepatectomy on 2016-2019. Volume of segments II and III were considered as FLR and assessed on pre- and post-embolization CT scans. Serum bilirubin above 50 µmol/L was used as a clinical marker of cholestasis. The degree of hypertrophy (DH) as percentual increase, and kinetic growth rate (KGR) as percentage/week, were used to assess PVE-induced hypertrophy. A total of 50 patients (31 CRLM, 19 pCCA) were included. The DH and KGR were similar in patients with pCCA and CRLM. Neither bilirubin levels before biliary drainage (pCCA patients) nor before PVE (entire cohort) were correlated with DH or KGR. For patients with pCCA, unilateral drainage in FLR induced a higher DH than bilateral drainage (6.7 [4.9-7.9] versus 2.7 [1.5-4.2] %, p = 0.012). After biliary drainage, ten patients with pCCA remained hyperbilirubinemic, but had comparable DH and KGR to patients with bilirubin levels below 50 µmol/L (DH: 5.6 [3.0-7.5] versus

5.7 [2.4-7.0] %, respectively, p=0.806; KGR: 1.7 [1.0-2.4] versus 1.9 [0.8-2.4] %/week, respectively, p=1.000). C-reactive protein levels before PVE were negatively correlated with DH and KGR in patients with pCCA (DH: ρ=-0.539, p=0.038; KGR: ρ=-0.532, p=0.041). Our findings indicate that there was no influence of cholestasis on hypertrophy of the FLR in patients undergoing PVE. Unilateral drainage in FLR was associated with higher liver hypertrophy than bilateral drainage. Elevated inflammation response appears to be associated with impaired liver growth in patients with pCCA.

Automated chyme reinfusion in intestinal failure patients with a temporary double enterostomy (TDE) restores intestinal function and protects against IFALD, but the mechanisms are incompletely understood. In **Chapter 6** we aimed to investigate whether beneficial effects of CR relate to functional recovery of enterohepatic signaling via the bile salt-FGF19 axis. Blood samples were collected from 12 patients, 3 days before, at start, and 1, 3, 5 and 7 weeks after CR initiation. Plasma FGF19, TBS, C4, citrulline (CIT), bile salt composition, liver tests and nutritional risk indices were determined. Paired small bowel biopsies prior to CR and after 21 days were taken and genes related to bile salt homeostasis and enterocyte function were assessed. CR induced an increase in plasma FGF19 and decreased C4 levels, indicating restored regulation of bile salt synthesis via endocrine FGF19 action. TBS remained unaltered during CR. Intestinal FXR was upregulated after 21 days of CR. Molar fractions of secondary and deconjugated bile salts were increased after CR, reflecting restored microbial metabolism of host bile salts. Furthermore, CIT and albumin levels were gradually rising after CR, while abnormal serum liver tests normalized after CR, indicating restored intestinal function, improved nutritional status and amelioration of IFALD. CR increased gene transcripts related to enterocyte number, carbohydrate handling and bile salt homeostasis. Taken together, beneficial effects of CR are partly mediated by recovery of the bile salt-FGF19 axis and subsequent homeostatic regulation of bile salt synthesis, contributing to treat/prevent IFALD.

In **Chapter 7** we provided a general discussion in the context of an important role of enterohepatic bile salt signaling in liver regeneration and intestinal failure, along with a perspective view on future studies and potential implications.