Research Article

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A synthetic biology-based device prevents liver injury in mice

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Background & Aims: The liver performs a panoply of complex activities coordinating metabolic, immunologic and detoxification processes. Despite the liver's robustness and unique self-regeneration capacity, viral infection, autoimmune disorders, fatty liver disease, alcohol abuse and drug-induced hepatotoxicity contribute to the increasing prevalence of liver failure. Liver injuries impair the clearance of bile acids from the hepatic portal vein which leads to their spill over into the peripheral circulation where they activate the G-protein-coupled bile acid receptor TGR5 to initiate a variety of hepatoprotective processes.

Methods: By functionally linking activation of ectopically expressed TGR5 to an artificial promoter controlling transcription of the hepatocyte growth factor (HGF), we created a closed-loop synthetic signalling network that coordinated liver injury-associated serum bile acid levels to expression of HGF in a self-sufficient, reversible and dose-dependent manner.

Results: After implantation of genetically engineered human cells inside auto-vascularizing, immunoprotective and clinically validated alginate-poly-(L-lysine)-alginate beads into mice, the liver-protection device detected pathologic serum bile acid levels and produced therapeutic HGF levels that protected the animals from acute drug-induced liver failure.

Conclusions: Genetically engineered cells containing theranostic gene circuits that dynamically interface with host metabolism may provide novel opportunities for preventive, acute and chronic healthcare.

Lay summary: Liver diseases leading to organ failure may go unnoticed as they do not trigger any symptoms or significant discomfort. We have designed a synthetic gene circuit that senses excessive bile acid levels associated with liver injuries and automatically produces a therapeutic protein in response. When integrated into mammalian cells and implanted into mice, the circuit detects the onset of liver injuries and coordinates the production of a protein pharmaceutical which prevents liver damage.

Keywords: Genetically engineered cells; Gene- and cell-based therapy; Liver disease; Regeneration; Synthetic biology; Synthetic gene circuits.

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Abbreviations: HGF, hepatocyte growth factor; PKA, cAMP-dependent phosphokinase A; CRE, cAMP-response element; P_{CRE}, CRE-containing synthetic mammalian promoter; SEAP, human placental secreted alkaline phosphatase; CREm, modified cAMP-response element; P_{CREm}, modified P_{CRE} variant; TUDCA, tauroursodeoxycholic acid; shGLP1, short human glucacon-like peptide 1; SBA, serum bile acid; CCl_4, Carbon tetrachloride; ANIT, alpha-naphthylisothiocyanate.

Introduction

The liver is associated with over 500 functions which include the clearance of the blood from toxic compounds, drugs and infectious agents, the control of blood fat and glucose levels, and the recovery, processing and conversion of digested food into metabolic energy [1]. The processing of digested food requires the production of bile and its major component, the bile acids. Bile acids are synthesized from cholesterol in the liver, secreted by the hepatocytes into bile canaliculi and stored in the gall bladder [2]. After each meal, bile acids are released into the duodenum to emulsify ingested fats and other lipophilic nutrients, reabsorbed in the terminal ileum and transported back to the liver via the portal vein, a process known as enterohepatic circulation [2,3]. Efficient clearance and recycling of bile acids from the portal vein ensures low bile acid levels in the peripheral circulation, which increase only marginally after each meal [2]. However, in patients suffering from diverse liver diseases such as cirrhosis, cholestasis, hepatitis and liver cancer, fasting blood bile acid levels are markedly increased due to the impaired hepatic clearance of bile acids from the portal vein [4–10]. Serum bile acid (SBA) levels therefore serve as sensitive indicator of liver disease [4–10]. Because of the minor daily meal-based fluctuations and their excessive levels during liver-associated pathologies, SBAs have been suggested to play a more prominent metabolic role exceeding the one of emulsifying nutrients [2,11].

Indeed, bile acids have recently emerged as versatile signalling compounds endowed with systemic endocrine function

[2]. Bile acids are ligands of several nuclear hormone receptors such as farnesoid X receptor (FXR) and G-protein-coupled receptors (GPCR) such as TGR5 through which they activate diverse signalling pathways that regulate triglyceride, cholesterol, glucose and energy homeostasis as well as their own synthesis, enterohepatic circulation, inflammation and liver regeneration [2,11]. In particular, bile acid-mediated activation of TGR5 coordinates renal clearance of bile acids, thereby preventing toxic bile acid overload [11,12]. In addition, pro-inflammatory mediators induce the production of hepatocyte growth factor (HGF), which has a potent cytoprotective impact on hepatocytes, triggers their proliferation and stimulates migration and proliferation of activated hepatic stem cells into the liver parenchyma, where the cells differentiate into mature hepatocytes [13–15].

Liver diseases are particularly difficult to diagnose as latent inflammations leading to critical fibrosis, irreversible cirrhosis and organ failure may go unnoticed as they fail to trigger any symptoms or significant discomfort [16,17]. To date, liver transplantation is the major treatment option for late-stage liver diseases [1]. However, because of the shortage of donor livers, as well as the significant risk associated with transplantation and life-long immunosuppression, a genetically engineered cell-based theranostic liver-protection device combining precise diagnosis of acute liver injuries with targeted therapeutic or hepatoprotective interventions may represent an attractive alternative.

Capitalizing on SBAs as a well-established biomarker for a wide variety of liver-associated pathologies [4–10], TGR5 as a liver injury-specific bile acid sensor and HGF as a promising protein therapeutic validated in human clinical trials [18–21], we have functionally linked these components using a synthetic biology-based design strategy to create a closed-loop synthetic gene network that detects the onset of liver injury, initiates HGF-mediated liver regeneration and prevents liver failure.

Materials and methods

Components of the liver-protection device

Comprehensive design and construction details for all expression vectors are provided in Table 1. The key components of the liver-protection device includes pBP2 for constitutive low-level expression of the human bile acid receptor TGR5 ($P_{hCMV^{*}-1}$-TGR5-pA) and pPB5, which produces human hepatocyte growth factor in a bile acid-responsive TGR5-dependent manner (P_{CREm}-HGF-pA).

Cell culture and transfection

Human embryonic kidney cells (HEK-293, ATCC: CRL-11268 [HEK-293T]), baby hamster kidney cells (BHK-21, ATCC: CCL-10), human fibrosarcoma cells (HT-1080, ATCC: CCL-121) and telomerase-immortalised human mesenchymal stem cells (hMSC-TERT, [22]) were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Basel, Switzerland; cat. no. 52100-39) supplemented with 10% (v/v) fetal bovine serum (FBS; Sigma-Aldrich, Munich, Germany; cat. no. F7524, lot no. 022M3395) or 10% (v/v) charcoal-stripped FBS (cFBS, Sigma-Aldrich; cat. no. F6765, lot no. 13C443) and 1% (v/v) penicillin/streptomycin solution (Sigma-Aldrich; cat. no. P4333). Wild-type Chinese hamster ovary cells (CHO-K1, ATCC: CCL-61) were cultured in ChoMaster® HTS (Cell Culture Technologies, Gravesano, Switzerland; cat. no. HTS-8) supplemented with 5% (v/v) FBS and 1% penicillin/streptomycin solution. Freestyle™ 293-F suspension cells (Life Technologies, Carlsbad, CA; cat. no. R79007) were cultivated in FreeStyle™ 293 Expression Medium (Life Technologies; cat. no. 12338018) supplemented with 1% penicillin/streptomycin solution and grown in 12 well plates or shake flasks placed on an orbital shaker (IKA KS 260 basic; IKA-Werke GmbH, Staufen im Breisgau, Germany; cat. no. 0002980200) set to 100–150 rpm. All cell types were cultivated at 37 °C in a humidified atmosphere containing 5% CO_2. All cell lines were transfected using an optimized polyethyleneimine (PEI)-based protocol [23]. For transfection of CHO-K1, HEK-293, BHK-21, HT-1080 and hMSC-TERT, 5×10^4 cells seeded per well of a 24 well plate 20 h before transfection were incubated with a transfection solution containing 0.55 µg plasmid DNA (for co-transfections equal amounts of plasmid DNA was used) and 2.2 µl of PEI (polyethyleneimine; MW40,000, stock solution 1 µg/µl in ddH_2O; Polysciences, Eppelheim, Germany; cat. no. 24765-2). For transfection of suspension FreeStyle™ 293-F cells, 1×10^6 cells seeded per well of a 12 well plate 1 h before transfection were incubated with a transfection solution containing 1.1 µg plasmid DNA and 4.4 µl PEI (1 µg/µl). The DNA/PEI transfection solution was mixed with 50 µl 150 mM NaCl, incubated for 15 min at 22 °C and added dropwise to the cells. Cell concentrations were profiled with a CASY® Cell Counter and Analyser System Model TT (Roche Diagnostics GmbH, Mannheim, Germany).

Animal experiments

Intraperitoneal genetically engineered cell implants were produced by encapsulating pHY74/pSP16 ($P_{hCMV^{*}-1}$-eYFP-pA/P_{CREm}-SEAP-pA)-, pPB2/pSP16 ($P_{hCMV^{*}-1}$-TGR5-pA/P_{CREm}-SEAP-pA)-, pPB2/pPB5 ($P_{hCMV^{*}-1}$-TGR5-pA/P_{CREm}-HGF-pA)- and pPB7 (P_{hCMV}-HGF-pA)-transgenic HEK-293 cells into coherent alginate-poly-(L-lysine)-alginate beads (400 µm; 200 cells/capsule) using an Inotech Encapsulator Research Unit IE-50R (Buechi Labortechnik AG, Flawil, Switzerland) set to the following parameters: 25 ml syringe operated at a flow rate of 450 units, 200 µm nozzle with a vibration frequency of 1,020 Hz and 1.1 kV for bead dispersion, stirrer speed at 4.5 units. 8 week-old female OF1 mice (oncins France souche 1, Charles River Laboratory, Lyon, France) were intraperitoneally injected with 2–5 × 10^6 cells (700 µl DMEM containing 1–2.5 × 10^4 capsules, 200 cells/capsule) and were treated with bile acids (cholic acid [0–160 mg/kg] or tauroursodeoxycholic acid [0–160 mg/kg], twice-daily intraperitoneal injections of 0–80 mg/kg; cholic acid [0–160 mg/kg] or deoxycholic acid [0–80 mg/kg], twice-daily intravenous injections of 0–80 mg/kg; cholic acid [0–160 mg/kg], tauroursodeoxycholic acid [0–40 mg/kg] or deoxycholic acid [0–160 mg/kg], twice or fourth-daily oral administration of 0–40 mg/kg), hepatotoxicants (1-Naphtyl isothiocyanate [75 mg/kg] or carbon tetrachloride [1 ml/kg], single oral dose) or olive oil (8 ml/kg, single oral dose). Blood samples were collected 48 h after treatment and the serum was isolated using BD Microtainer® SST tubes according to the manufacturer's instructions (centrifugation for 5 min at 10,000 g; Becton Dickinson, Plymouth, UK; cat. no. 365967). All mice were kept on a standard diet (5 kcal % fat; Janvier S.A.S., Le Genest-Saint-Isle, France) unless indicated otherwise. After completion of the experiments, the animals were sacrificed and their liver collected for histological analysis. All experiments involving animals were performed according to the directives of the European Community Council (2010/63/EU), approved by the French Republic (no. 69266309 and no. 69266310; project no. DR2013-01 (v2)) and carried out by Ghislaine Charpin-El Hamri and Marie Daoud-El Baba at the University of Lyon, Institut Universitaire de Technologie (IUTA), F69622 Villeurbanne Cedex, France.

Histology

Two days after implantation of microencapsulated pPB2/pPB5-, pPB2/pSP16- or pPB7-transgenic cells and subsequent oral administration of a single dose of ANIT or olive oil, the mice were sacrificed and their livers were explanted. Five representative liver slices (2–3 mm thick) of each animal were fixed in 0.1 M sodium cacodylate buffer (pH7.4, 540 mOsm; Sigma-Aldrich, St. Louis, USA, cat. no. CO250) containing 2.5% (v/v) glutaraldehyde (AGAR Scientific Ltd., Stansted, UK, cat. no. R1010) and stored at 4 °C until further processing. Tissue blocks of approximately 1 mm^3 were cut, rinsed in 0.1 M sodium cacodylate buffer (3 × 10 min, pH 7.4, 340 mOsm) and post-fixed in 0.1 M sodium cacodylate buffer containing 1% (w/v) osmium tetroxide (Electron Microscopy Sciences, Hatfield PA, USA cat. no. 19100) at 4 °C for 2 h. The samples were then washed in 0.1 M sodium cacodylate buffer (3 × 10 min, pH 7.4, 340 mOsm), dehydrated by serial incubation in ddH_2O containing increasing concentrations of ethanol (70%, 80%, 90%, 96%, 99%, 100%) and embedded in EPON 812 (Fluka, Sigma-Aldrich, Buchs, CH, cat. nos. 45245-45347). One µm thick liver sections were prepared using glass knives, stained with toluidine blue (Fluka, Buchs, CH, cat. no. 89640) and analysed by light microscopy (100×–630× magnification, Imager M2, Zeiss, Jena, De). At least five animals per treatment group were analysed.

Statistics

Results are expressed as mean ± SEM. Statistical significance of datasets was evaluated by a two-tailed, unpaired Student's *t* test using Graphpad Prism V6.0d. $p < 0.05$ was considered significant.