Participant Abstracts
for
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Metabolic reprogramming in livers of mice with chronic liver disease

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Metabolic zonation of healthy liver lobules is extensively studied. However, little is known how chronic liver disease (CLD) influences lobular zonation. To bridge this gap, we studied metabolic zonation in two mouse models of CLD: repeated intoxication with carbon tetrachloride (CCl4; 1 gram/kg) twice a week up to one year, and bile duct ligation (BDL) for 21 days. Disease progression and its impact on metabolic zonation was analysed by histopathology, immunohistochemistry, image analyses, RT-PCR, as well as RNA-sequencing (RNA-seq) techniques. Repeated CCl4 intoxication triggered centro-centro bridging pattern of fibrosis that was detected already at 2 months. At late stages (1 year), progression to cirrhosis with presence of regenerative and neoplastic nodules was detected. RNA-seq analysis revealed downregulation of the pericentral and upregulation of the periportal genetic programs, respectively. Furthermore, immunohistochemistry analysis revealed spatio-temporal alterations of the pericentral and the periportal proteins. At the early stage (2-6 months), the pericentral proteins, e.g. CYP450 enzymes and glutamine synthetase, showed decreased diameter of the positive area and centro-centro bridging pattern. At the late stage (1 year), the expression of the pericentral proteins was almost completely lost. In contrast, the territory of the periportal proteins, e.g. the urea cycle enzymes, was increased time-dependently to cover almost the entire liver parenchyma. To check whether the loss of pericentral gene expression occurs because CCl4 intoxication targets the pericentral hepatocytes, the same analyses was done after BDL; a model of periportal fibrosis. Interestingly, the pattern of periportalization of the liver lobule was also consistent in the BDL model. Biostatistical analysis of the RNA-seq data suggested that periportalization of the liver lobule in CLD was due to a loss of Wnt/β-catenin signalling pathway. In order to check the functional consequences of this altered zonation, mice on day 21 post BDL or on one year of repeated CCl4 intoxication were challenged with 200 mg/kg acetaminophen (APAP). Interestingly, both mouse models were almost completely resistant to APAP intoxication. In conclusion, CLD strongly alters zonation of the liver lobule where the periportal genetic program becomes dominant, forming a 'periportal-like' lobule.

Mass Spectrometry Lipidomics technology in LiSyM

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Shotgun mass spectrometry analysis of lipids is one of the major techniques used in the lab of Andrej Shevchenko from Pillar I.

One of the directions of work in LiSyM was the application of shotgun lipidomics to human liver biopsies. Samples were previously histologically characterized, including the evaluation of NAFLD development stage, and screened for known and suspected NAFLD mutation risk markers: PNPL3, MBOAT7, TM6SF2, HSD17B13, SERPINA Z and SERPINA S. Meta-data collected from the same patients included BMI, age, disease stage, comorbidities and medication.

MS analysis of the full cohort, comprising 367 liver biopsies has been recently completed. Variations of the measurements across all batches is considered acceptable (e.g. QC standard deviation was 15% for neutral lipids and 24% for phospholipids). The obtained lipidome underwent post-acquisition filtering, resulting in the dataset of 316 lipids from 19 lipid classes measured in 367 independent liver samples.

The PNPLA3 mutation-based analysis of NAFLD and NASH groups reveals the distinct changes nearly exclusively in TAG species in the samples where homozygous mutation was present compared to the samples with no mutation in PNPLA3. The MBOAT7 mutation-specific changes affect exclusively PI species in NAFLD and NASH groups. This analysis allows suggesting the presence of different development and manifestation mechanisms of NAFLD and NASH depending on the mutation present.

Another work direction in Shevchenko lab is the Laser Capture Microdissection (LCM) coupled to Shotgun Lipidomics. We developed an approach to quantify lipids from distinct regions of interest (ROIs) in mouse liver by combining LCM and fluorescence microscopy with top-down shotgun lipidomics. LCM was applied to collect specific ROIs (hepatocytes next to pericentral and periportal regions) to investigate differences in spatial lipid distribution from mice liver. The zonation was previosly typically characterized at the protein and transcriptional level, however full-lipoidome profiles in physiological norm and pathological conditions remained elusive and could not be revealed by mass spectrometry imaging typically relying on a few readily ionisable molecules. Liver-specific ROIs were detected by immunofluorescence using glutamine synthetase as a pericentral marker combined with DAPI stained nuclei to identify periportal regions. For the analysis, we collected 0.5 mm2 of 20 µm thin native cryo-sections roughly corresponding to 500-600 cells from pp and pc part of the liver lobule. In separate experiments with a model sample having controlled lipid composition we established that lipids isolation by LCD is non-destructive, quantitative and does not bias lipid compositions. We were able to quantify 16 lipid classes, including free Chol, Chol-esters and TAG, covering more than 200 lipid species from periportal and pericentral zones. Comparison of full-lipidome profiles revealed that compositional differences are restricted to a few specific molecules of ceramide and sphingomyelin lipid classes, while in contrast to some previous reports, the lipoidome is not globally affected by metabolic zonation. These findings have major implications for our understanding of lipid metabolism in liver.

Structural and functional multi-parametric analysis of chronic liver disease, liver injury and repair using complementary non-invasive imaging techniques

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Structural and functional multi-parametric analysis of chronic liver disease, liver injury and repair using complementary non-invasive imaging techniques

The objective of our work is the longitudinal multi-parametric analysis of chronic liver disease (CLD), liver injury and repair using complementary non-invasive imaging techniques. Protocols were adapted and optimized for the longitudinal multi-parametric analysis of the liver at high resolution 7 T MRI. Currently, we are investigating the morphological, structural and functional alterations during CLD progression using the CCl4 model of liver fibrosis.

Besides T1- and T2-weighted morphological imaging, dynamic sequences with two different contrast agents are applied to assess hepatocyte function and macrophage activity. Diffusion-weighted imaging provides information about the cellularity and the deposition of extracellular matrix. Fat-selective sequences are used to assess steatosis. The experiments are currently ongoing.

A Bayesian Network Approach to Biomarker Discovery of NAFLD

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Large epidemiological cohort studies offer excellent conditions for biomarker discovery due to the broad nature of the collected data, ranging from socioeconomic and clinical parameters to different OMICs data. They thereby cover lots of possible influences as well as confounders. However, the heterogeneous, incomplete, and high-dimensional data need to be approached by appropriate methods. Above all, efficient multivariate and integrative methods are still rare. Bayesian Networks (BNs) are probabilistic graphical models that can visualize and quantify complex associations among many variables simultaneously. However, BN learning from data using machine learning is highly computationally expensive. We thus propose to combine the network learning with hierarchical variable clustering. We show that by this, the dimension of the model can be significantly reduced while still capturing the relevant associations. As the quality of the resulting model depends highly on the chosen variable grouping, we propose to adaptively refine the clustering in inaccurate areas of the network while learning. This process is automated and can take into account a variable of interest (e.g., a specific disease). The resulting probabilistic biomarker network may then be very detailed around the variable of interest, and coarser in less relevant regions. We compare the proposed algorithm to state-of-the-art BN learning algorithms using simulated data as well as data from the Study of Health in Pomerania (SHiP), a large epidemiological cohort study. Taking non-alcoholic fatty liver disease as an example, we show that the resulting model can be used for both, individual risk prediction as well as biomarker discovery.

A PBPK model for simulating the effect of liver cirrhosis on drug PK

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In silico trial simulations are gaining importance in pharmaceutical development. Among other reasons for this, computational modelling enables simulating the impact of specific physiological properties in special populations on drug exposure. Thus, changes in drug efficacy and safety can be mechanistically analysed. Physiologically based pharmacokinetic (PBPK) modelling is recognized as an important tool to integrate heterogeneous experimental data into a whole-body context. In particular, PBPK modelling allows relating knowledge on physiological changes in patients to observed alterations in drug pharmacokinetics (PK).

Here, we present the development of a PBPK model for cirrhotic patient populations which allows the analysis of the impact of functional changes on drug PK in such individuals. Liver cirrhosis is a progressive disease which is associated with severe pathophysiological alterations. Several potential physiological changes such as decreased liver enzyme activity, ascites, porto-systemic shunting, decreased albumin production and many more can have an impact on drug PK. As a first step, ascites was successfully integrated into a PBPK model for cirrhotic patients. Simulations of a test compound adequately described the concentration-time profile in the plasma as well as in the ascitic fluid. As a second step, the decrease in activity of several cytochrome P450 enzymes such as CYP1A2, CYP3A4, CYP2C19 and CYP2E1 was included in the PBPK model. Here, we focused on describing continuous disease progression according to the Child-Pugh grading system. The model is currently evaluated using test compounds with different physicochemical properties and specific clearance pathways. After this initial evaluation, further pathophysiological alterations will be taken into account. In the next step, PK data of the LiSyM drug cocktail trial in cirrhotic patients will be analysed using the developed model. Finally, the model should allow improved in silico trial simulations in cirrhotic patient cohorts as such significantly supporting regulatory submissions for novel compounds.

Hedgehog Signaling as a mediator between liver and adipose tissue

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Background

In recent years it has become apparent that the morphogenic Hedgehog (Hh) pathway is not only active during embryonic development, but also in adult organs, controlling metabolism and maintaining homeostasis. The liver in particular as a metabolically active organ with many functions in energy homeostasis is of interest, especially since active Hh signaling could be detected in hepatocytes.

Crosstalk between the liver and tissues of the periphery such as the adipose tissue (AT) has long since been known. Prior experiments have revealed that inactivation of Hh in hepatocytes leads to morphogenic changes in several tissues, one of which is the AT. The mechanism and implications of such a Hh-dependent crosstalk between the tissues remains to be elucidated.

Methods

Primary hepatocytes as well as the different types of AT – visceral (VAT), subcutaneous (SAT) and brown (BAT) – were isolated from two mouse strains with specific inactivation of Hh signaling in hepatocytes. Morphologic and biochemical characterization of AT by immunohistochemistry including analysis of adipocyte size as well as by molecular biological methods such as qPCR was performed.

Results

Mice with an inactivation of Hh signaling in hepatocytes have a distinct phenotype in AT: an increase of all types of AT in both sexes as well as a changed distribution of cell size could be

The interaction of Hedgehog and mTor signaling in healthy hepatocytes

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Introduction

The Hedgehog (Hh) pathway is one of the central morphogenetic pathways. It plays a key role in embryonic development and is a regulator of regeneration and stem cell function in adult tissues. Abnormal Hh Signaling contributes to different diseases like hepatic steatosis and cancer. Studies showed various interactions of the Hh pathway and the signaling of the mechanistic target of rapamycin (mTor) in different cancer types. The mTor pathway is an important mediator between nutritional signals and their appropriate cellular responses. However, the mechanisms behind the interaction of Hh and mTor Signaling are poorly understood, particularly in healthy tissue. In our studies, we demonstrate synergistic effects of both pathways in controlling healthy liver metabolism.

Methods

Primary hepatocytes from C57Bl6/N mice were treated with the Hh inhibitor Cyclopamine, the mTorC1 inhibitor Rapamycin, the mTorC1/C2 inhibitor Torin and the combinations thereof. The cells were analyzed using Western Blots, qPCR and Seahorse technology.

Results

Our experiments show synergistic inhibition by Cyclopamine and Rapamycin of p70S6 phosphorylation, a downstream kinase of mTorC1. Cyclopamine alone and Rapamycin alone have no or only a weak influence on the phosphorylation, respectively. When we repress mTorC1 and C2 with Torin, we see the same effect as with Cyclopamine and Rapamycin. However, the addition of Cyclopamine to Torin has no further influence on the phosphorylation state. In addition, we see this effect also in the phosphorylation state of Rictor, an mTorC2 component. Moreover, although the inhibition of the Hh pathway alone has no impact on the functionality of mitochondria, our Seahorse analyses show an increased inhibition of the electron transport chain in cells incubated with Rapamycin and Cyclopamine compared to Rapamycin alone.

Conclusion

We suppose that the minor impact of Rapamycin on mTor pathway activity is an effect of the feedback mechanism via mTorC2. Therefore, our results suggest a possible interaction of the

Differential hepatic mRNA and miRNA expressions in a novel mouse model of bacterial infection (BI)-related acute-on-chronic liver injury (ACLI)

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Background and Aims: Bacterial infection (BI) is a common acute trigger leading to acute-on-chronic liver failure in humans. Here we aim to establish a novel BI-ACLI model by LPS injection in a knock-out mouse with chronic hepatobiliary injury (Abcb4-/-) to mimic disease conditions of ACLF.

Methods: 15-weeks-old C57BL/6J (N=16; wild-type, wt) and Abcb4-/- (N=16; knock-out, ko) mice were treated with a single dose IP injection of either LPS (4 mg/kg) or saline (0.9% NaCl). qRT-PCR and stem-loop qRT-PCR were used to assess relative hepatic mRNA and miRNA expression. miRNAs 26a, 29a, 122, 143, 146, 192m and Let7c, Crp, Tnf-α, Rantes, Tgf-β, Tlr4, Mcp1 and interleukins (IL) 2, 6, 10, 17 and 22 were evaluated by 2-ΔΔCt method.

Results: LPS resulted in a dramatic change in Mcp1, IL-10, IL-6, Rantes and Tnf-α expression with 200-, 63-, 45-, 38- and 31-fold increases in wt mice, and relatively moderate upregulation of Crp (4-fold), IL-2 (5-fold), and Tlr4 (6-fold). Corresponding effects were also observed in ko mice, whereby upregulation of IL-6, IL-10, Tnf-α, Tlr4 and Mcp1 was not significant. In line with the differential regulation of IL-22 and IL-17, where IL-6 alone is sufficient for IL-22 induction and high levels of IL-6 and Tgf-β are required for IL-17 upregulation, Tgf-β and IL-17 did not differ among groups. More profound induction of Rantes (118-fold vs. 38-fold increase, p=0.037), IL-22 (75-fold vs. 5-fold, p<0.01) and Crp (9-fold vs. 4-fold, p=0.026) was present in ko mice as compared to wt. No IL-22 was detected in NaCl-treated mice, whereas it was stimulated by LPS in both genotypes with a 7.3-fold higher upregulation (p<0.01) in ko mice. Ko mice displayed higher basal expressions of miR-122 (p=0.029), miR-192 (p=0.037), and miR-143 (p<0.01). Upregulation of miR-122 and miR-192 and repression of miR-143 and miR29a were evident upon LPS treatment without significant differences between genotypes. miR-26a, miR-146 and miR-Let7c, which were previously identified as markers for liver fibrosis and inflammation, were neither affected by Abcb4 deficiency nor LPS challenge.

Conclusions: We propose a novel approach to model BI-ACLI in Abcb4-/- mice with differential expressions of hepatic cytokines, chemokines and miRNAs after LPS challenge. We speculate that IL-2, IL-22 and Rantes could specifically trigger inflammatory cascades with harmful consequences on disease progression in chronic hepatobiliary diseases.

Characterization of molecular alterations in chronic liver diseases using quantitative mathematical modeling of HGF-induced hepatocyte proliferation

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In acute-on-chronic liver failure (ACLF) the liver's ability to regenerate is impaired. In patients with an underlying chronic liver disease an acute event induces severe damage that can lead to death within four weeks due to organ failure. Mice fed with a western diet containing high fat and high sugar levels develop steatosis and can be used to analyze chronic liver disease progression.

A mechanistic modeling approach was applied to define molecular alterations in hepatocyte proliferation. Hepatocyte growth factor (HGF) regulates proliferation of hepatocytes by inducing MAPK and PI3K signaling pathways. To define the dynamics of HGF induced signal transduction, primary mouse hepatocytes were analyzed by quantitative immunoblotting. First, quantitative data from healthy hepatocytes was used to calibrate a dynamic mathematical model based on ordinary differential equations describing HGF induced signal transduction. Furthermore, kinetics of HGF induced signaling pathways in hepatocytes from western diet induced fatty livers were analyzed and compared to healthy mouse hepatocytes by quantitative immunoblotting. The results indicate similarities but also major differences in the signaling of fatty hepatocytes. The calibrated quantitative mathematical model is able to describe the effects of the western diet on hepatocyte signal transduction and can help to understand mechanistic changes leading to chronic liver diseases.

Evaluating the benefit of individual patient data for physiologically based pharmacokinetic (PBPK) simulations

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In this work, personalized physiologically-based pharmacokinetic (PBPK) models for idazolam and caffeine were built. The goal of the study was to evaluate whether incorporation of additional information about the study volunteers improves the agreement of the volunteer-specific PK simulations with corresponding PK measurements.

As a starting point, the midazolam PBPK model was fitted to the mean concentration of all volunteers. The model was simulated with the biometrics of the PK-Sim® reference individual (caucasian, male, 30 years, 73 kg, 1.76 m). In a second step, the biometrics (height, weight, age, sex) of the virtual volunteers were set to the reported biometrics of the study volunteers and compared to the respective PK data. Finally, the expression of the drug metabolizing enzyme CYP3A4, which was the most sensitive parameter of the midazolam PBPK model, was fitted to the observed data.

Inclusion of biometric information (height, weight, age, sex) of study individuals did not improve the predictive performance of midazolam PBPK model simulations. The individual PK data are already well described by a simulation with a reference individual (80% of the data being in the 2-fold range). The presented approach contributes to a profound understanding of PK variability which is crucial for optimization of efficacy and safety in clinical science.

Simulating Lattice-Free Agent-Based Multicellular Models with the TiSim Framework

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In recent years, mathematical and computational models have become an essential tool to understand the complexities of tissue organization processes. Capturing the mechanisms acting on different scales of description of these models and forming them into a model capable of being simulated on a computer is often a resource-consuming effort, requiring skills spanning different scientific disciplines. While still a fairly intricate task, the creation and parametrization of such models can be facilitated by specialized computational frameworks.

As such a framework, TiSim offers interfaces to parametrization, GUI and CLI deployment and eases the reuse of existing models already implemented in TiSim.

With the upcoming release, we provide several use cases of TiSim as a framework and the a glimpse of its capabilities as a workbench software for virtual experiments involving multiple scales (such as diffusion and intracellular pathways coupled with cell fate) not only for center-based models, but also for models with explicitly deformable cells [1].

[1] P Van Liedekerke, J Neitsch, T Johann, K Alessandri, P Nassoy, D Drasdo; Quantitative cell-based model predicts mechanical stress response of growing tumor spheroids over various growth conditions and cell lines, PLoS Computational Biology, 2019, https://doi.org/10.1371/journal.pcbi.1006273

Small GTPases in Steatosis: The case of Rab18

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Rab18 is a member of the Ras/Rab-family of small GTPases and is known to interact with lipid droplets (LDs). Yet, the role of Rab18 on LDs and mechanisms of localization remain largely unknown. We investigate the role of Rab18 in the liver, since LD formation in hepatocytes is the most conspicuous phenotypic marker of liver steatosis.

We show that like other small GTPases, Rab18 localization and enrichment on LDs is regulated by irreversible prenylation, and an acylation cycle of dynamic palmitoylation. Palmitoylation deficient mutants of Rab18 lacking this acylation cycle mislocalized to the ER in HepG2 cells, with no enrichment on LDs.

We perturbed the acylation cycle responsible for localization to the LD's membrane using palmitoylation and depalmitoylation inhibitors. Fluorescent Recovery after Photobleaching (FRAP) showed that Rab18 recruitment to LDs was impaired in cells treated with a palmitoylation inhibitor, while Rab18 depletion was correspondingly impaired in the presence of a depalmitoylation inhibitor. Thus, inhibition of the acylation cycle through small molecule inhibitors can be used to affect the enrichment of Rab18 on LDs.

We demonstrate that under a siRNA-mediated Rab18 knockdown, LD size increased in oleic acid-induced HepG2-cells. Overexpressing wildtype Rab18 rescued the wildtype phenotype in cells under Rab18 knockdown, whereas overexpression of mislocalized Rab18 mutants did not. Rab18 localization to the LD's membrane therefore appears to prevent droplet enlargement, antagonizing large LD development.

We surmise that the manipulatable localization of Rab18 activity directly affects LD growth kinetics. This rational approach to lipid droplet biology in the liver reveals Rab18 to be a plausible target for pharmacological intervention for preventing pathological progression of steatosis.

Lipidomic Analysis of Pediatric Non-Alcoholic Fatty Liver Disease

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Objectives: Nonalcoholic fatty liver disease (NAFLD) represents the most common chronic liver disease in children and adolescents. Fatty acid (FA) composition - especially in the liver - seem to play an important role in the pathogenesis and disease course. Essential omega-6 (n-6) and omega-3 (n-3) FA are polyunsaturated fatty acids (PUFA) that are metabolized into bioactive lipid mediators via cytochrome P450 enzymes (CYP450). These lipid mediators include epoxyeicosanoid acids that have shown to have anti-inflammatory and anti-steatotic effects in experimental NAFLD. Epoxyeicosanoid acids can further be hydrolyzed into biologically less active dihydroxyeicosanoid acids by soluble epoxide hydrolase (sEH). The aim of this study was to characterize FA profiles of pediatric patients with biopsy proven NAFLD and to examine potential changes of n-3 and n-6 derived lipid mediators in respect to histological disease severity.

Methods: We performed a comprehensive LC-MS based lipidomic analysis of fatty acids and lipid mediators in 40 pediatric patients with biopsy-proven NAFLD in liver tissue and whole blood samples. Analysis of these lipidomic profiles were conducted with respect to histological characteristics. Statistical analysis was performed using Kruskal-Wallis-Test or one-way analysis of variances (ANOVA) with Games-Howell post hoc test.

Results: Lipidomic analysis revealed distinct FA profiles in liver tissue and blood samples. Hepatic lipid content of all FA ($p<0.05$), saturated fatty acids (SFAs; $p<0.01$) and mono-unsaturated fatty acids (MUFAs; $p<0.05$) significantly increased with higher steatosis grade, while the relative n-3 contribution to total FA (n-3 index) significantly decreased with higher steatosis grade ($p<0.05$). In addition, hepatic epoxyeicosanoid acids, especially epoxydocosapentaenoic acids (EDPs; $p<0.001$), epoxyeicosatetraenoic acids (EEQs; $p<0.05$) and epoxyeicosatrienoic acids (EETs; $p<0.01$) significantly increased with higher steatosis grades while their corresponding stem FA were not altered. These findings were reflected in whole blood samples, although only EEQ content did reach significance ($p<0.05$). We also found that the ratio of 9,10 dihydroxyoctadecanoic acids (DiHOME) to epoxyoctadecanoic acids (EpOME) which is an indicator of sEH activity significantly decreased with higher steatosis grades ($p<0.05$).

Conclusion: FA profiles showed typical features of the western diet including high intake of SFAs and low n-3 FA intake in our patient cohort. The data indicates that biologically active epoxyeicosanoid acids increase with higher steatosis grades, most likely due to a decrease of sEH activity, which results in a limited conversion into biologically less active dihydroxyeicosanoid acids. We therefore speculate that the increase of epoxyeicosanoid acids with higher steatosis grades represents a protective mechanism in the development of NAFLD.

Modeling the Interaction of Wnt and Hh in the Adult Liver in Mice and Humans

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The Hedgehog (Hh) and Wnt/β-Catenin (Wnt) cascades are well-studied signaling pathways that are important for controlling many processes in embryogenesis and adult homeostasis. Both pathways have been found to be active in adult liver where they are thought to coordinate various metabolic pathways. Based on in vitro and in vivo knockout experiments in mice and in humans we propose a simple model for the interaction of the two pathways. The model results are consistent to the observed experiments showing a pericentralization of liver tissue upon Wnt dominance and a periportalization of liver tissue upon Hh dominance. This suggests that the two pathways act complementary to each other and are involved in establishing zonated regions of liver function.

Benchmarking functional genomics tools on simulated and real single-cell RNA-seq data

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With the emergence of high-throughput transcriptome profiling, many tools have been developed to extract functional and mechanistic insight from bulk expression data. With the advent of single-cell RNA sequencing, it is possible to do such an analysis for single cells. However, this technology has its own limitations and characteristics such as drop-out events, low library sizes and a comparative large number of samples/cells. In this study, we perform benchmark studies on in silico and in vitro data to explore whether functional genomics tools developed for bulk data can be applied on single-cell RNA-seq data. We focus on the tools PROGENy and DoRothEA that estimate pathway and transcription factor (TF) activities, respectively. For the in silico study, we simulate single cells from TF/pathway perturbation bulk RNA-seq experiments. Our simulation strategy guarantees that the information of the original perturbation is preserved while the characteristics of single-cell RNA-seq data are introduced. Benchmarking the performance of both tools on the simulated single cells reveal a comparable performance to the original bulk data. In addition, we conduct an in vitro study on selected real single-cell RNA-seq datasets. We show that the functional characterization of these data sets is in agreement with existing knowledge.

With regard to the investigation of chronic liver diseases, functional analyses results inferred from bulk and single-cell RNA-seq data can be combined. This would provide a much more detailed picture of the general disease progression and which liver cell types play a pivotal role during progression.

PK-DB: A Pharmacokinetics Database for Individualized and Stratified Modeling

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We present PK-DB, a database for the representation of pharmacokinetics data from clinical trials and pre-clinical research. Data is either curated from the literature or from available raw data. The main focus of PK-DB is to provide high-quality pharmacokinetics data in combination with required meta-information for computational modeling and data integration, i.e., (i) characteristics of studied patient collectives and individuals; (ii) applied interventions (dosing, route, …); and (iii) measured pharmacokinetics information and time courses. Important features are the representation of experimental errors and variation, the representation and normalization of units, annotation of information to biological ontologies, and calculation of pharmacokinetics information like apparent clearance, half-life, or area under the curve (AUC) from time course data.

We demonstrate the value of PK-DB by a stratified meta-analysis of pharmacokinetics studies for caffeine curated from the literature and by integrating data with a physiologically based pharmacokinetic (PBPK) model for codeine.

CYP2E1 recovery is associated with a pericentral fibrosis pattern after repeated CCl4 insults

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Fibrosis is a consequence of repetitive liver injuries, e.g. upon viral infection, alcohol consumption, malnutrition or hepatotoxicants. Based on the etiological factor, liver fibrosis develops in different patterns and presents as septal in toxic injuries[1], biliary in cholestatic diseases[2], bridging upon hepatitis virus infections or pericellular in case of alcohol consumption. The mechanism behind the generation of the different patterns is still elusive. We aim to define (a) molecular driver(s) of fibrosis pattern formation. Mice were exposed to acute or repeated doses for 6 consecutive weeks of carbon tetrachloride (CCl4). Morphologically, patterns of fibrosis and metabolizing enzymes, namely CYP2E1, were analyzed in immunostaining datasets. We found that the pattern of CYP2E1+ hepatocyte recovery after acute insult is similar to the observed toxic-induced septal fibrosis. This similarity suggested that the spatial pattern of CYP2E1 might indicate the location where the fibrosis forms. To study this hypothesis, we developed a dynamic activator-inhibitor system, where the activator is a diffusible protein released from the central vein to promote the CYP2E1 signaling while the inhibitor is a diffusible protein released from the portal vein to inhibit the CYP2E1 signaling. Currently, this model can partially capture the observed patterns of CYP2E1 and extracellular matrix (ECM) upon chronic liver injuries suggesting that the pattern of CYP2E1 may indeed be a key factor in determining the location of fibrotic streets despite likely not the only one. We are currently extending our model by further mechanisms. These include, but are not limited to i) crosstalk between activated hepatic stellate cells and liver sinusoidal endothelial cell differentiation; ii) The dialogue between endothelial cells lining the hepatic veins and hepatocyte metabolic zonation; iii) Presence of so far unknown diffusible inhibitor in the portal compartment, i.e. a bile duct driven factor. In the next step, we target the WNT/β-Catenin pathway (CYP2E1 regulator) by monoclonal antibodies against R-spondin1, 2 or 3 in fibrosed liver, and iv) the mechanical role of ECM deposited by HSCs, all finally integrated in a spatial-temporal model established to mimic regeneration after administration of a single dose of CCl4[3].

[1]Hammad S, Braeuning A, et al. A frequent misinterpretation in current research on liver fibrosis: the vessel in the center of CCl4-induced pseudolobules is a portal vein. Arch Toxicol. 2017 Nov;91(11):3689 – 3692.

[2]Hammad S, Cavalcanti E, et al. Galunisertib modifies the liver fibrotic composition in the Abcb4Ko mouse model. Arch Toxicol. 2018 Jul;92(7):2297 – 2309.

[3]Hoehme S, Brulport, M, et. al.. Prediction and validation of cell alignment along microvessels as order principle to restore tissue architecture in liver regeneration. Proc. Natl. Acad. Sci. (USA), 107(23), 10371 – 10376.

Identifying alterations of proliferation signaling in chronic liver disease

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Healthy hepatocytes can undergo several rounds of cell division to repair injured liver tissue. In acute-on-chronic liver failure (ACLF) this regenerative capacity of the liver is impaired and affected patients often die due to organ failure. Mice fed with Western Diet develop steatosis and serve as a model for the analysis of chronic liver disease progression.

In order to understand the underlying processes and identify key mechanisms altered during liver regeneration in ACLF patients mechanistic modeling is applied. Based on quantitative time-resolved signaling data of healthy primary mouse hepatocytes a dynamic model is established. It integrates the two main signaling pathways involved in proliferation, MAPK and PI3K signaling, both regulated by the hepatocyte growth factor (HGF). After calibration of the model to this healthy scenario it is applied to the disease situation. Signaling dynamics in western diet hepatocytes show significant differences, which can be deduced to their origin using mathematical modeling. Thereby, we are able to determine mechanistic differences between the healthy and the disease stage on a molecular level. The developed model is able to describe the effects of the western diet on hepatocyte signal transduction and gives new insights in the progression of chronic liver disease.

Analysis of macrophage population in the liver of WD-treated mice

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Due to changes of life style the non-alcoholic fatty liver disease (NAFLD) is currently evolving to an important cause of chronic liver diseases, especially in western countries, were it meanwhile achieves a prevalence of 20-30 %. The main risk factors for developing non-alcoholic fatty liver disease (NAFLD) are overweight, diabetes, and metabolic syndrome. NAFLD is the basis for the development of a chronic inflammatory condition also referred to as non-alcoholic steatohepatitis (NASH). It is known that in the pathogenesis of NASH activation of inflammatory cells such as neutrophils or macrophages plays a critical role. However, the molecular mechanisms involved in their activation are not fully understood. Apart from this the differential contribution of the different inflammatory cell populations is unclear and in how far changes of macrophage polarization plays a role during disease development and progression has not been studied in detail. An in depth understanding and a detailed characterization of these processes may contribute to identify molecular patterns indicating an increased risk of disease progression or development of acute on chronic liver failure.

The different aspects outlined above will be addressed in the present project by a system biology approach. In particular the characterization of time-resolved evolution of macrophage-polarization during development of NAFLD and NASH is within the focus of the project. Own data indicate that the intercellular communication between the different cell populations of the liver and in particular between hepatocytes and macrophages has a strong impact on function and differentiation/polarization of the respective cell type. Therefore, the impact of fat accumulation in hepatocytes on the intercellular communication between hepatocytes and macrophages and the resulting changes of macrophage function is also addressed.

Additionally, staining of CD68+ and CD11b+ macrophages has been established to further characterize resident or recruited macrophages. In the fatty liver a stage of disease dependent local accumulation of macrophages in proximity to fat loaded hepatocytes was observed, also termed as „crown-like structures".

Moreover, in an ongoing working package using FACS based cell separation time dependent changes of the transcriptome and proteome are assessed in cooperation with the group of Ursula Klingmüller in sessile (F4/80+/CD14low) and recruited liver macrophages (F4/80+/CD14high). F4/80+ cells of the non-parenchymal fraction from WD-fed mice were analysed according to their CD14 signal intensity by flow-cytometry-based cell sorting. These studies showed that the majority of macrophages detected are F4/80+ and CD14high and therefore appear to be recruited to the liver. The transcriptome of tissue macrophages isolated from livers of animals fed with WD for 20 weeks showed that along with high expression of CD14 the cells show major changes of their transcriptome in the course of NAFLD development. To further complete these data additional time resolved series of WD-induced NAFLD have been performed. Currently additional series are performed with longer feeding periods of up to 40 weeks. First results showed that after 20 weeks of feeding with WD also markers for recruited monocytes/macrophages like Ly6C, CCR2 or CX3CR1 were upregulated.

Liver sinusoidal endothelial cells, their constitutive HGF release and response to IL-6-cluster-signaling.

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Liver sinusoidal endothelial cells (LSECs) have unique capabilities to coordinate the immune response in the liver. Recently we showed that LSECs are crucial for activated T cell-mediated liver failure through antigen cross-presentation. The resulting fulminant hepatitis leads to massive liver damage, but also a fast and complete recovery. Therefore we propose that LSECs are also orchestrating liver regeneration in these cases.

We theorize that LSECs orchestrate early stages of Liver regeneration in case of a fulminant hepatitis by increasing the availability of matured HGF. HGF is produced in an inactive Pro-Form that binds to extracellular matrix proteins and is proteolytically cleaved, releasing the bio-active Form into the environment.

Rather we found that LSECs are potent suppliers of HGF in vitro without additional stimuli. In addition to that, they are responsive to IL-6-cluster-signaling in a dose-dependent fashion, by increasing the production of HGF. IL-6 cluster-signaling appears to be exclusive to LSECs in the liver tissue and neither hepatocytes nor liver resident macrophages increase HGF production upon stimulation. Initial proteome analysis revealed the upregulation of pathways connected to angiogenesis as well as proliferation, indicating a so far undiscovered IL-6-cluster-signaling derived response of LSEC.

We conclude that LSECs have the potential to be key initiators for liver regeneration in response to an immune system derived liver damage, by the integration of IL-6 cluster-signaling to further the availability of active HGF in the vicinity of the damage. The LSECs capacity to induce HGF in vitro presets itself to be unique in liver tissue and further research needs to be done in regards to possible in vivo implications of that pathway.