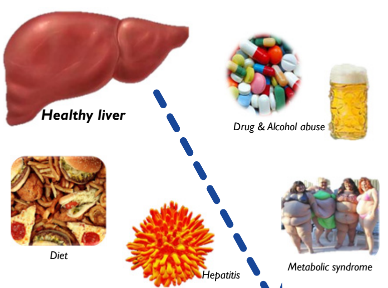
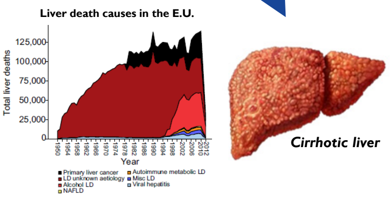
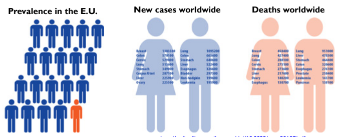
l'ivr Cell biology lab

Causes of liver disease



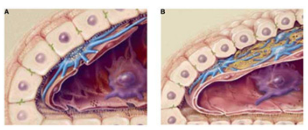


Prevalence of liver disease



http://onlinelibrary.wiley.com/doi/10.3322/caac.20107/pdf

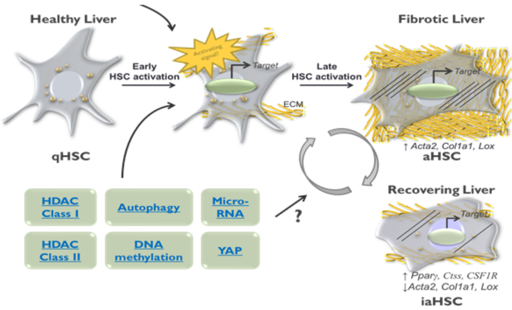
Liver architecture



Liver disease is caused by several insults. Chronic injury results in cirrhosis and loss of organ function. The most common cause of liver related deaths is liver cancer, which is the general end-stage of any chronic liver disease. In the E.U. 6% of the population suffers from a chronic liver disease and worldwide after lung and stomach cancer, liver cancer is the third leading cause of cancer related death. During liver disease a multitude of cells are damaged and this leads to production of scar tissue by the hepatic stellate cells (HSCs). Ultimately, excessive deposition of scar results in organ failure and death.

CURRENT PROJECTS @ LIVR

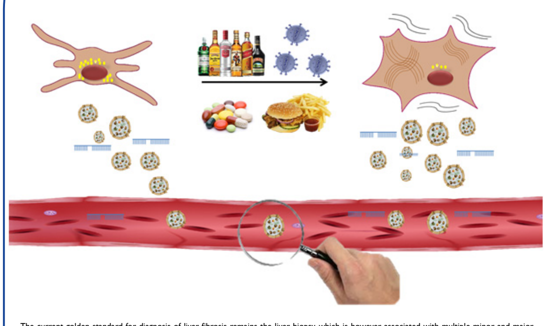
Mechanisms of fibrosis development and resolution



For many years it has been known that Hepatic stellate cell (HSC) activation is a key step in the development and progression of liver fibrosis. At first, acute damage to the liver causes the HSCs in the liver to transdifferentiate from a quiescent (qHSC) into an activated cell (aHSC). The quiescent cells are the main storage site for vitamin A in the body and the presence of vitamin A containing lipid droplets allows isolation of the cells based on density and/or autofluorescence. The activated HSCs on the other hand are the main source for scar tissue production in chronic liver disease. They have a myofibroblast phenotype, characterized by cell proliferation, migration and contraction. More recently, it was described that HSC inactivation occurs during recovery of disease in reversible rodent models for fibrosis. These inactivated HSCs (iaHSC) display an intermediate phenotype between quiescent and activated HSCs.

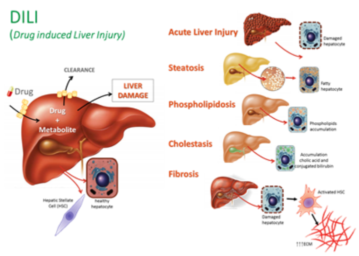
Because of their essential participation in the scar production, HSCs have been proposed as important targets for anti-fibrotic therapy. In order to develop more efficient treatment strategies, we try to get a better understanding of how the early phase of HSC activation is regulated and to investigate mechanisms involved in the inactivation of the cells.

Identification of HSC-specific biomarkers for fibrosis staging



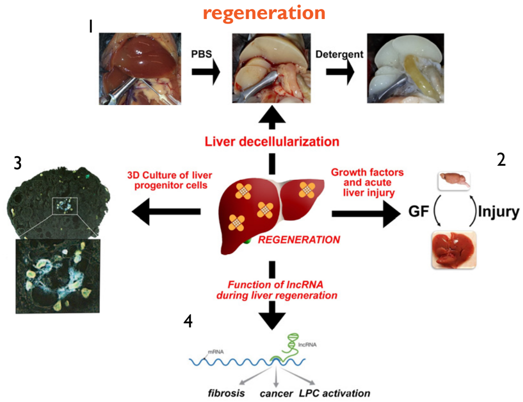
The current golden standard for diagnosis of liver fibrosis remains the liver biopsy, which is however associated with multiple minor and major complications. Other non-invasive scoring systems have been developed, but till date, none of these are able to detect early events of liver fibrosis development, nor are they capable of differentiating between the different stages of fibrosis progression. As hepatic stellate cells (HSCs) remain the major cell type in the liver fibrosis process, we turn to this cell for novel methods of diagnosis. Moreover, we hypothesize that extracellular vesicles (ECVs) derived from HSCs could harbor biomarkers of liver fibrosis. ECVs are small membrane-derived structures that can be divided into 3 subtypes (exosomes, microvesicles, apoptotic bodies), which carry cargo consisting of proteins, lipids, miRNA, lncRNA, …HSC-specific ECVs derived from the circulation of patients with liver fibrosis will be extracted and analyzed for their potential diagnostic content.

Generation of *in vitro* liver disease models



Hepatic organoids of human cells and mouse freshly isolated cells, were developed and optimized in the lab as an attempt to reproduce hepatocyte-HSC interactions specially in the case of drug-induced fibrosis. With this purpose we had observed that organoids can be kept in their healthy state (good hepatocyte functions and quiescent HSC) up to 21 days if not stimulated. Once challenged to single and repeated exposure to reference compounds, they can developed fibrotic features that resemble the fibrosis in the liver. Ongoing work is using the same model to try to reproduce features of other drug-induced liver diseases (DILI) such as cholestasis, phospholipidosis and steatosis.
Liver adverse effects are a common reason for drug discontinuation and market withdrawal. The use of co-cultures improves predictability of potential side effects of drugs in development and reduces the number of animals needed for reliable drug testing.

Role for liver progenitor cells during liver regeneration



Different studies on liver regeneration. 1) Liver decellularization: differently injured livers are decellularized and investigated based on their extracellular matrix and their influences on in vitro cultured liver cells. 2) Liver-pituitary gland axis is important during acute or chronic liver injury. Certain growth factors play an important protective effect on hepatocytes. 3) Organoids containing liver progenitor cells (LPCs) are used to develop a system that can mimic LPC activation in vitro. 4) Long non-coding RNAs (lncRNA) have an important function in the regulation of gen expression. Therefore we study which lncRNA is enriched and what is their function during liver fibrosis, LPC activation and cancer.

Frequently used Techniques @ LIVR

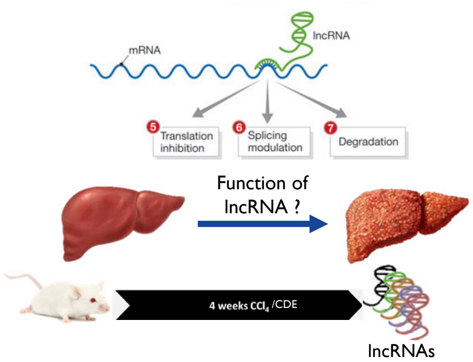
Isolation of liver cells
- FACS
- 2D culture
- 3D culture

RNA analysis: QPCR
Protein analysis :
- WB
- IF
- IHC

Functional assays:
- Cell viability
- Cell proliferation
- Collagen cross-linking

Proposed projects for internships @ LIVR

Long non-coding RNAs in chronic liver disease

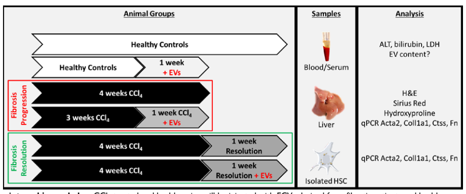


Long non-coding RNAs (lncRNA) are important regulators of gene expression. They can inhibit translation, modulate splicing and regulated the degradation of mRNAs. For now, there is very little known about the role of lncRNAs during chronic liver injury. Therefore, we will study which lncRNA is highly expressed during chronic livery and investigate their function. We will first look into RNAseq data of human livers to collect all lncRNAs differently regulated in fibrotic/alcoholic patients and confirm this in mice treated with CCl_4 of CDE diet.

Pro- and anti-fibrotic effects of circulating extracellular vesicles in human liver disease

Circulating extracellular vesicles (ECVs) are small membrane derived structures which are shedded by cells in both physiological and pathological conditions. The presence of ECVs has been already proven in multiple body fluids such as urine, cerebrospinal fluid, and blood. During the process of liver fibrosis and cirrhosis, an enhanced presence of blood-circulating ECVs can be seen. Additionally, we observed changing miRNA-cargo in these circulating ECVs during liver fibrosis, supporting its potential as a fibrosis biomarker.

Although more insight in the functionality of these circulating ECVs is gained in the cancer research field, their importance during liver fibrosis and cirrhosis largely remains unknown. During the internship at the LIVR lab, you will analyze the importance of these circulating ECVs. This will be done by transplantation of isolated ECVs from healthy and fibrotic patients, into healthy, fibrotic and recovering animals. This to investigate their potential capacity to modify fibrosis development.



Internship workplan. CCl_4-treated and healthy mice will be injected with ECVs derived from fibrotic patients and healthy individuals. Direct after treatment, or after 1 week of resolution, the mice will be sacrificed and blood, total liver, and hepatic stellate cells will be isolated. Pro-or anti-fibrotic effects of injected ECVs will be analyzed by ALT measurement, H&E staining, Sirius Red staining, hydroxyproline assay and qPCR analysis.

For more information visit http://livr.vub.ac.be/

Prof. Leo van Grunsven leo.van.grunsven@vub.ac.be
Dr. Inge Mannaerts inge.mannaerts@vub.ac.be
Doctoraatstudent Stefaan Verhulst stefaan.verhulst@vub.ac.be
Masterstudent Liza Dewyse liza.dewyse@vub.ac.be