Normalization of LSEC function attenuates and reverses hepatic fibrosis

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Date:	10-6-2021
Course:	Bachelor's Thesis Life Sciences WBBY901-05

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Abstract

Chronic liver diseases (CLDs) have been a major burden on society, and they still are. Annually they are responsible for 3.5% of the deaths worldwide. Liver cancer is responsible for 31.3% of these deaths, but most of them are related to cirrhosis (68.7%), which is the most common end-stage of CLDs. Common processes amongst many CLDs are inflammation and fibrosis. The most ideal treatment for CLDs is the treatment of the underlying etiology. This has been successful to some degree in the treatment of CLDs that are of viral origin. However, when this is not available, other therapies are needed. One of them is the use of antifibrotics. Until today there has been no approved antifibrotic for the treatment of CLDs for human use yet. However, there are a couple of them in clinical trials. The search for antifibrotics has mainly been focused on two cell types, the hepatic stellate cell (HSC) and the portal myofibroblast (PMF). However, fibrosis is a multicellular process that depends on the interplay between all types of tissue-resident cells. A cell type that has been neglected for quite some time, but recently gained more attention in the search for antifibrotics in CLD, is the liver sinusoidal endothelial cell (LSEC). LSECs have unique morphological and functional properties that make them crucial in maintaining tissue homeostasis, but also in disease. Therefore, this thesis will focus on the role of LSECs under normal and pathological conditions. It will highlight their importance and provide a basic understanding of their involvement in pathology of hepatic fibrosis and CLDs. Moreover, it will evaluate the potential of therapeutics targeting LSECs that normalize LSEC function in disease by highlighting some of the most recent and influential findings regarding therapeutics targeting LSECs that show great potency in the attenuation and reversal of hepatic fibrosis.

Keywords: LSECs, CLDs, hepatic fibrosis, antifibrotics

1. Introduction

1.1 The ongoing shift in the burden of Chronic liver diseases

CLDs have been a major burden on society for the past decades and they still are. Between 2012 and 2017 there was an increase of 11.4% in liver-related deaths, resulting in an annual mortality number of 2.14 million. Liver cancer accounted for 38.3% of these deaths, whereas cirrhosis accounted for 61.7% (Paik et al, 2020). Combined they are responsible for 3.5% of the deaths worldwide (Asrani et al, 2019). In the year 2017, 1.5 billion people were estimated to be affected by CLDs of which 60% originated from non-alcoholic fatty liver disease (NAFLD), 29% from hepatitis B virus (HBV), 9% from hepatitis C virus (HCV) and 2% from alcoholic liver disease (ALD). Currently a shifting pattern is observed within the total burden that these types of CLDs cause. Due to the success of vaccination and antiviral treatment programs, a decrease has been observed in the prevalence and complications of HBV. However, an increase was seen in the prevalence of acute HCV. On the other hand, HCV can nowadays be treated relatively well with antiviral therapy. NAFLD and ALD on the contrary, cannot so easily be treated since their underlying etiology is non-viral. This is one of the main reasons why we desperately need antifibrotic treatments. The prevalence of NAFLD and ALD is increasing. This can be attributed to the western lifestyle that is often associated with obesity, metabolic syndrome and high intake of alcohol (Moon et al, 2020).

1.2 Common characteristics of the CLD pathophysiology and spatial orientation of the cells involved

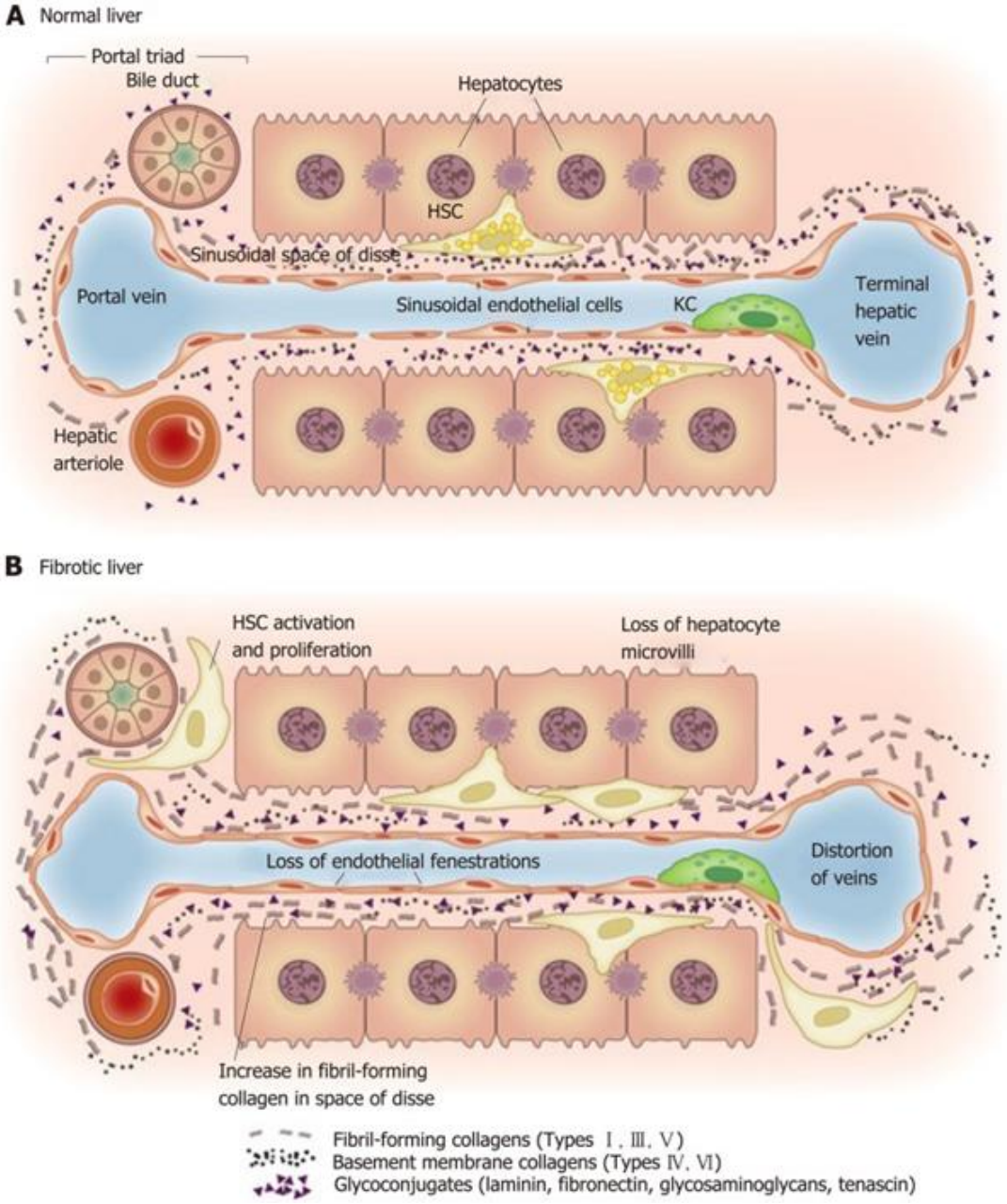
Many CLDs have a different pathophysiology, however almost all of them share common characteristics that are highly significant in the progression of CLDs, namely chronic inflammation and hepatic fibrosis. Chronic inflammation usually precedes hepatic fibrosis and affects the normal wound healing process that is initiated upon damage to the liver. It can promote fibrosis that is characterized by deposition of high amounts of extracellular matrix (ECM), that leads to tissue stiffness and remodeling of the tissue (Aydın & Akçalı, 2018). Prolongation of liver fibrosis can rapidly lead to cirrhosis, which is the end-stage of many CLDs. It is characterized by necrosis and degeneration of hepatocytes, replacement of normal liver tissue by fibrotic scar tissue and liver failure (Zhou et al, 2014). The most common complication caused by CLDs is portal hypertension, which is also the leading cause of death of CLDs (May et al, 2011). The underlying cause of portal hypertension is the increased vascular resistance of the hepatic sinusoids due to processes such as hepatic fibrosis, LSEC dysfunction and HSC activation (McConnel & Iwakiri, 2018).

There are many different types of cells that play a role in all of these processes that each have their own distinct role. Some of them are considered more important than others. Hepatic fibrosis is a multicellular process in which Kupffer cells (KCs), HSCs, LSECs and hepatocytes have a complex interplay with each other. This interplay is also influenced by the fibrotic microenvironment (Natarajan et al, 2017). The main cellular type that has been investigated in these pathological processes are the hepatic stellate cells (HSCs). In hepatic fibrosis they lose their quiescent state and become activated. When they are activated, they start to proliferate and secrete an excess of ECM (Jiao et al, 2009). This ECM accumulates in the space of Disse, which is where the HSCs reside. The space of Disse is located between the basolateral site of hepatocytes and the anti-luminal side of the liver sinusoidal endothelial cells (LSEC) [fig-1A]. The space is filled with permeable connective tissue, which makes the exchange of molecules between the portal venous blood and hepatocytes possible, supporting the main function of the liver (Higashi et al, 2017).

The main function of the liver is metabolism of molecules and foreign substances before they reach the systemic circulation. The hepatocytes contain all sorts of metabolizing enzymes which are mainly

the cytochrome P450 enzymes. When a drug is orally administered, it is first metabolized by the liver, usually resulting in a decreased concentration of the drug in the systemic circulation. This process is referred to as first-pass loss and must be considered when designing orally administered drugs. Moreover, the hepatocytes produce bile, which is important in the breakdown of fat molecules and storage of certain fat-soluble vitamins such as vitamin A (Kalra et al, 2020). Vitamin A is stored in the HSCs. HSCs have shown to lose their capability to store vitamin A in hepatic fibrosis (Freund & Gotthardt, 2017). Bile is secreted into the duodenum by the gallbladder upon the release of cholecystokinin, which is stimulated when eating. Bile is composed of several substances including bile acids and bile salts. They are involved in a process called emulsification that is responsible for (1) the digestion of lipid molecules to micelles that store fats and fat-soluble vitamins in a compact matter, and (2) the suspension of fat particles in water, which makes them more accessible for pancreatic lipases (Chen & Cassaro, 2020). The micelles have a hydrophobic tail and a hydrophobic head, making them water soluble and transportable to the surface of the small intestine. When they have arrived, they break down and have their cargo absorbed in the small intestine via passive diffusion (Cheryan, 2020). Another key function of the liver is the acute phase response. The acute phase response is initiated upon host infection, inflammation or injury, which causes the production of cytokines via the innate immune response. These cytokines, produced by all sorts of inflammatory cells, activate different processes that try to reinstate homeostasis and minimize the damage upon initiation of the repair response in case of tissue injury. However, the cytokines also stimulate the upregulation of certain plasma proteins called acute phase proteins as C-reactive protein and mannoses, that are involved in clearance of infections. These proteins carry out their function via the opsonization of dead cells, dying cells or bacteria. It basically makes these cells better recognizable for immune cells that ultimately clear the infection (Jain et al, 2011). Normally, the mannose and C-reactive protein levels are much lower. The liver is responsible for the production of almost all plasma proteins, with the most abundant being albumin. Albumin is important for maintaining blood volume and pressure by contributing to the plasma osmotic pressure. It is also involved in the transport of hydrophobic substances as fatty acids and steroid hormones. Furthermore, albumin increases the half-life time of hydrophobic drugs by binding to them and in this way keeping their plasma levels high. Other produced plasma proteins are globulins and to a lesser extent fibrinogens. Globulins are important for the transport of iron, lipids and vitamins. These globulins are produced by the liver. However, there is also a subset of globulins that are involved in the immune response. These are called antibodies and are produced by plasma cells. Lastly, fibrinogens are important for blood clot formation (Biga, 2019).

Cells that have always been very important in the functioning of the liver are the LSECs. They form the outline of the hepatic sinusoids, which is where the high-pressure, oxygen-rich blood from the hepatic artery is mixed with low-pressure, oxygen-low blood from the portal vein (Lautt, 2009). The hepatic artery, portal vein and central vein, which collects oxygen-low blood from the liver lobules, belong to the systemic vasculature and are composed of vascular endothelial cells, a type of endothelial cell that is distinctly different from LSECs (Strauss et al, 2017). LSECs have large fenestrae and lack a basement membrane making it easy for molecules to exchange between the portal venous blood and the space of Disse (DeLeve, 2015). LSECs are becoming more and more acknowledged over the past few decades to have an emerging role in the progression of CLDs and liver cancer (Wilkinson et al, 2020).



(Figure retrieved from Hernandez-Gea & Friedmann, 2011)

Fig. 1 LSEC microenvironment during physiological conditions homeostasis and hepatic fibrosis.

(A) LSEC microenvironment during physiological conditions. LSECs are fenestrated and separated from the hepatocytes by the space of Disse, which makes the exchange of substances between the portal venous blood and the hepatocytes possible. **(B)** Changes in LSEC microenvironment during hepatic fibrosis. Upon injury, LSECs become defenestrated and lose their capability of keeping HSC quiescent, resulting in the secretion of large amounts of ECM in the space of Disse. This impairs the normal exchange of substances between the portal venous blood and hepatocytes and contributes to the development of portal hypertension. (Hernandez-Gea & Friedmann, 2011)

1.3 The main focus of current clinical trials and their lack of LSEC investigation

The research for treatment of CLDs mainly focuses on two different aspects. One of them is treatment of the underlying etiology of the disease, which has been shown successful in the reversal

of liver fibrosis for HBV and HCV, using the antiviral therapies. The other one is treating the fibrotic process by trying to attenuate or reverse hepatic fibrosis. However, until now there have been no antifibrotic therapies approved for human use (Guo et al, 2020). The focus of present antifibrotic treatment studies has been inhibition of myofibroblast activation, stimulation of apoptosis, deactivation of myofibroblasts, the usage of liver-protective agents reducing liver damage, stimulation of ECM degradation and treatment of the underlying etiology (Shu et al, 2021). Currently investigated antifibrotics have shown to be effective in the halting of progression of hepatic fibrosis, however they are still far from the ultimate goal of achieving total reversal (Chang & Li, 2020). The main cell types that have been targeted by the research community are the activated HSC and PMF. The HSC is a precursor of myofibroblasts that when activated starts to secrete large amounts of ECM, eventually causing fibrosis. Together with the activated PMF, activated HSCs and activated PMFs are responsible for most of the ECM deposition during toxic liver injury, with the overall consensus of the HSC being the predominant cell contributing to the total myofibroblast population. However, there is still ongoing debate whether the current view on the origin of liver myofibroblasts is as it is now. Limitations in the experimental quantification of the origin of myofibroblasts derived from HSCs and PMFs might mask the contribution of smaller cell populations that also act as a source of myofibroblasts in liver injury such as bone marrow derived mesenchymal stem cells and fibrocytes. An ongoing controversy is that fibrocytes form a much larger percentage in the myofibroblast pool in hepatic fibrosis (Kisseleva, 2017). Moreover, it is hypothesized that endothelial cells, hepatocytes and cholangiocytes also contribute to the myofibroblast pool after undergoing the process of endothelial or epithelial mesenchymal transition. However, definite in vivo evidence of these processes still has to be uncovered (Dewidar et al, 2019). What is interesting is that in a CCL4-induced fibrotic mouse model, activated HSCs are by far (87%) the most abundant myofibroblast. However, in a BDL-induced fibrotic mouse model, which is a cholestatic form of injury, activated PFs are the main source of myofibroblasts in the onset of liver injury (>70%). Over time this percentage decreases as after a few days HSCs become activated and a part of the myofibroblast population (Iwaisako et al, 2014).

Although targeting myofibroblasts has shown great potential in halting the progression of hepatic fibrosis, it has not been as effective in reversing hepatic fibrosis and is mainly considered as a therapy to control the disease and not to resolve it (Chang & Li, 2020). However, normalization of LSEC function in vivo and in vitro has shown great potential in the attenuation an reversal of hepatic fibrosis. LSECs are the first cells that sense the damaging stimuli, mainly because of their proximity to the portal venous blood, and have. Characteristics of LSECs upon damage are defenestration and capillarization [fig-1B] (Lafoz et al, 2020). Capillarization is a process in which the LSECs secrete a high amount of basement membrane proteins such as laminin, collagen IV and nidogen. Co-localization of these three proteins is associated with formation of a basement membrane and capillarization of LSECs (Chen et al, 2019). Defenestration and capillarization affect the high permeability of LSECs and thereby the bidirectional exchange of molecules between the portal venous blood and the liver parenchyma. Moreover, the change in the LSEC phenotype makes them lose their capability of keeping HSC in a quiescent state, which has been identified as an initial trigger for hepatic fibrosis (Lafoz et al, 2020). Defenestrated and cappilarized LSECs start to secrete large amounts of transforming growth factor beta (TGF-β) and platelet derived growth factor (PDGF) that drive the activation of the HSCs and their deposition of large amounts of ECM (Cheng et al, 2021). It as well results in a decrease of nitric oxide (NO) production by LSECs, which has a crucial role in keeping HSCs quiescent (DeLeve, 2015). Despite the increasing attention that LSECs receive for their emerging role in the progression of CLDs and liver cancer, the number of clinical trials targeting LSECs to attenuate or reverse hepatic fibrosis are still limited. Recent studies of in vitro and rodent models have shown great potential of drugs that target LSECs, thereby effectively attenuating and reversing

hepatic fibrosis and CLDs (Ma et al, 2021). Compounds such as statins and nuclear receptor agonists are making their way into the field of LSEC-targeted antifibrotics, which show promising results regarding attenuation and reversal of hepatic fibrosis. To better understand how these compounds are working and interfere with hepatic fibrosis, a general knowledge is needed of LSECs in their normal and fibrotic microenvironment. This will be further elucidated in the following section.

2. The niche of LSEC in normal and fibrotic microenvironment and their interaction with their neighboring cells

2.1 LSEC morphology and their functions

The liver cell population is divided into two different categories: parenchymal cells (30-40%) and non-parenchymal cells (60-70%). LSECs are part of the non-parenchymal cells and encompass around 50% of the total non-parenchymal cell population. The hepatocyte is the most common cell in the liver and is the main parenchymal cell. (Seo et al, 2016). LSECs lack a basement membrane and have highly permeable fenestrations that allow for bidirectional exchange of substances between the portal venous blood and liver parenchyma. (Sørensen et al, 2015; Lafoz et al, 2020). The fenestrations are approximately 50 – 200 nm in diameter and cover around 2 – 20 % of the LSEC surface. They are observed to be co-localized in groups of 10 – 100 forming so-called liver sieve plates, or they can be located individually across the LSEC surface [fig-2] (Warren et al, 2010). Examples of factors that can influence the size and number of fenestrations are blood pressure, hormones, drugs or the amount of ECM (Lafoz et al, 2020). The human endothelium can be entitled as being heterogeneous based on their basement membrane being continuous or discontinuous and on the presence of fenestrations. A continuous endothelium is found in most veins, arteries and capillaries of the skin, brain, heart and lungs. This endothelium does not contain fenestrations and has tight junctions that can protect for example the brain from toxins. However, a continuous endothelium with fenestrations can be seen in the glomeruli of the kidney, choroid plexus, gastrointestinal tract, exocrine and endocrine glands. Tissues with this type of endothelium have usually an increased role in filtration or trans-endothelial transport. Lastly, the discontinuous endothelium is characterized by fenestrations and a poorly structured or no basement membrane. The LSECs are the main example of such endothelium, although this type of endothelium is also present in the bone marrow and spleen (Félétou, 2011). Next to LSECs being the most permeable endothelial cells of the human body, they also have the highest endocytotic capacity of all human cells. This supports the metabolic function of the liver since endocytosis assists in the total extraction of substances from the blood and makes it more efficient. The extraction of substances is therefore not only limited by simple diffusion across the fenestrae but is complemented by the endocytotic capacity of LSECs (Knolle et al, 2016).

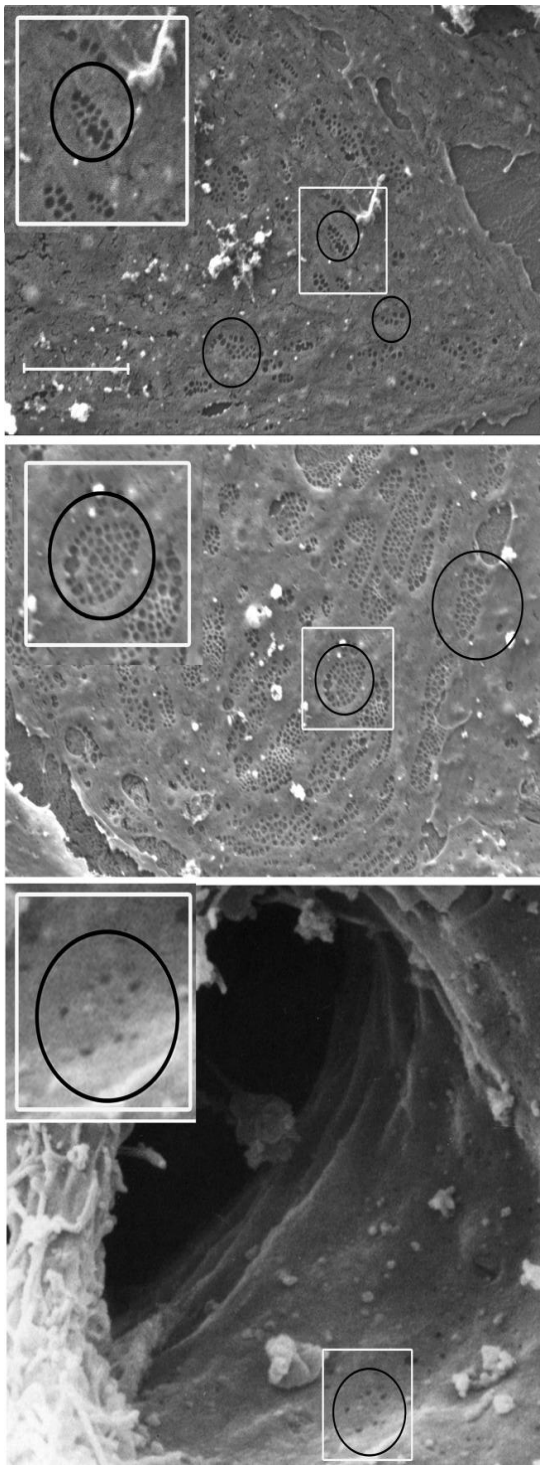


Fig. 2 Scanning electron microscopy of rat LECs.

upper panel, photomicrograph of a rat peritubular LSEC (magnification 5,000x) containing on average 8 fenestrae per sieve plate (encircled). Middle panel, photomicrograph of a rat centrilobular LSEC (magnification 5,000x) containing on average 24 fenestrae per sieve plate (encircled). Note that although the peritubular LSEC shows on average less fenestration per sieve plate, the average size of the peritubular fenestrations is larger compared to the centrilobular fenestrations. Lower panel, scanning electron photograph of a human liver sinusoid opening into the central vein (magnification 8,500x). LSEC fenestrations forming sieve plates (encircled) are rare in this transitional zone. An enlarged copy of the white rectangles is illustrated on the top left of each panel for a clearer view. (DeLeve, 2015)

(Figure retrieved from DeLeve, 2015)

2.2 Signaling cascades and LSEC secretions to maintain liver homeostasis

LSECs are involved in a complex interplay with hepatocytes, HSCs and KCs. All of these cell types have the capacity to secrete cytokines and growth factors that can influence the secretions of each other. This is also referred to as the tissue microenvironment. (Marrone et al, 2016; Natarajan et al, 2017). Another function of LSECs is that they maintain a low vascular tone, thereby making it possible to have a high exchange of substances with the liver parenchyma. LSECs do this by the production of nitric oxide (NO), which is a vasodilator (Poisson et al, 2017). Although LSECs maintain a low blood

flow, there is still a significant amount of shear stress present in normal tissue homeostasis due to the narrow diameter of the capillaries. Increasing microenvironment shear stress has shown to increase the NO synthesis by LSECs (Natarajan et al, 2017). However, when there is a continuous high level of shear stress as for instance in liver cirrhosis, Kruppel-like factor 2 (KLF2) is induced. KLF2 is involved in the upregulation of NO production [fig-3], but also in the downregulation of vasoconstrictors such as endothelin-1 (Gracia-Sancho et al, 2010; Poisson et al, 2017). Endothelin-1 is upregulated in patients with cirrhosis (Soydemir et al, 2020). In LSEC dysfunction, LSECs are no more able to regulate the fine balance between vasodilators and vasoconstrictors as in a healthy microenvironment. Dysfunction of this balance mediated by LSECs is often in co-expression with LSECs being unable to keep the HSCs in a quiescent state. In hepatic fibrosis this imbalance mainly results in a vasoconstrictive, pro-fibrotic environment caused by a decrease in vasodilators as NO and an increase in vasoconstrictors as endothelin-1. When in hepatic fibrosis, endothelin-1 is released from LSECs and acts in a paracrine manner on HSCs, it makes them produce endothelin-1 themselves and promotes their vasoconstriction resulting in an excess of ECM deposition. This excess of ECM contributes to the stiffness of the organ, and moreover increases the vascular tone contributing to portal hypertension, which is detrimental for the progression of CLDs (Ezhilarasan, 2020). In normal tissue homeostasis NO produced by LSECs is able to keep HSCs in a quiescent state by preventing the formation of a contractile microenvironment (DeLeve, 2015). Maintaining LSEC function is therefore associated with preventing progression of hepatic fibrosis and CLDs, promoting a healthy balanced tissue homeostasis.

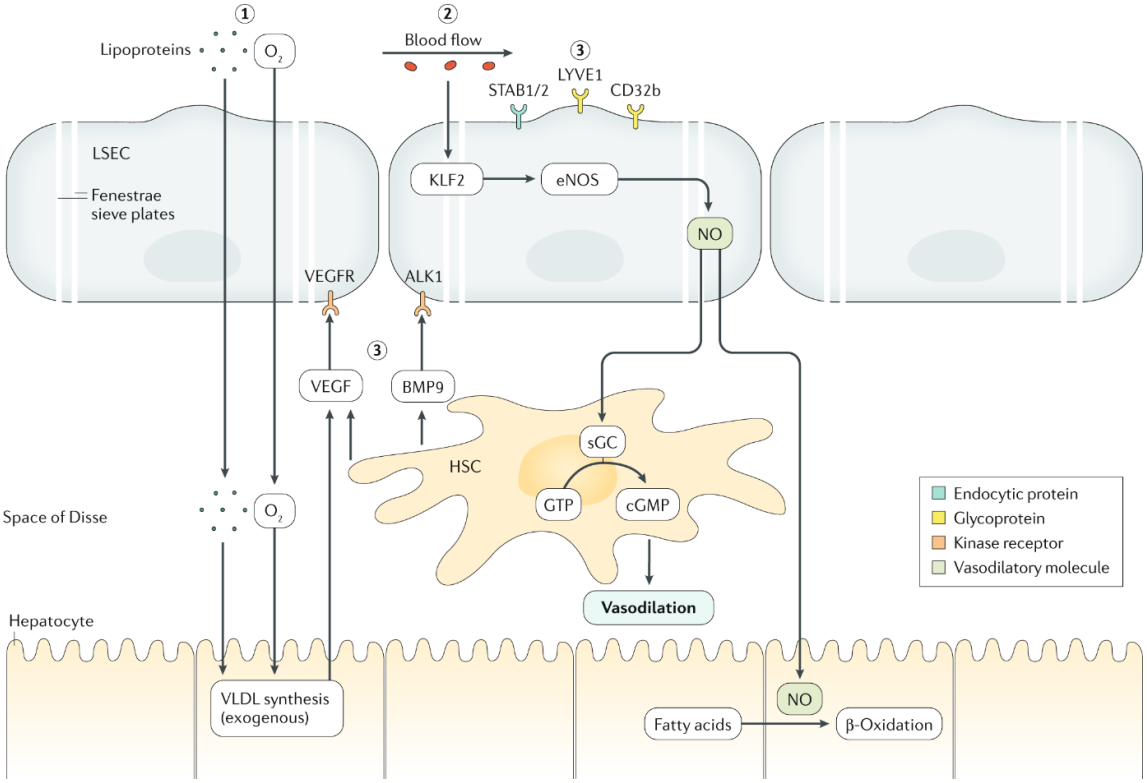
Another signaling molecule important in the interplay of the main cells involved in hepatic fibrosis and maintenance of tissue homeostasis mediated by LSECs, is vascular endothelial growth factor (VEGF). VEGF is produced by HSCs and hepatocytes and regulates LSEC phenotype via NO-dependent and NO-independent pathways. In the NO-dependent pathway, VEGF binds to its receptor, thereby stimulating endothelial nitric oxide synthase (eNOS) to produce NO. NO activates a downstream signaling cascade that works via soluble guanylate cyclase (sGC). sGC converts guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP) resulting in the activation of protein kinase G that phosphorylates targeted proteins, causing vasodilation. The NO-independent pathway still has to be uncovered (Ma et al, 2021; DeLeve, 2015). Interestingly, when activated HSCs were cultured in the presence of LSECs and VEGF, they returned to their quiescent phenotype. However, inhibition of eNOS prevented the return of activated HSCs to quiescent HSCs. Furthermore, when the LSEC phenotype was not maintained, they were not able to reverse the activated HSC to a quiescent state (DeLeve et al, 2008). Lastly, shutting down VEGF signaling in a transgenic animal model, has shown to cause the loss of LSEC fenestrae, independent of damage to the liver. The loss of fenestrae resulted in HSC activation and portal hypertension. Restoring VEGF signaling resulted in the reversing of portal hypertension (May et al, 2011). This shows that the signaling of VEGF stimulating NO production is very important in maintaining LSEC function and phenotype and that possible interventions in this pathway could be effective in reversing hepatic fibrosis and CLDs. This will be further elucidated in section 3.

2.3. LSECs have scavenger and immune receptors important for liver function

As indicated before, LSECs have the highest endocytotic capacity of the human body. This endocytotic process involves many different receptors which are also called scavenger receptors such as the collagen-α-chain/mannose receptor and the hyaluronan/scavenger receptors including LYVE1, stabilin-1 and stabilin-2 [fig-3]. They are involved in the endocytosis of all sorts substances including collagens, glycoconjugates with terminal mannose, oxidized and acetylated low-density lipoprotein (LDL), tissue plasminogen activator, and other ECM components as nidogen and

hyaluronan (DeLeve, 2015). Other receptors that LSECs have apart from these receptors and the earlier described VEGFR, are pattern recognition receptors (PRRs) that are involved in immunological responses by the liver. PRRs are a specific type of receptor that bind to pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs). Well-known examples of PRRs are the toll-like receptor 4 (TLR4) and CD14 that can bind to for instance a well-known PAMP, lipopolysaccharide (LPS) which is part of the outer membrane of gram-negative bacteria (Pandey et al, 2020). Historically, the general view had always been that KCs were responsible for the uptake of LPS, however in mice it was found that KCs are responsible for the uptake of 25% of the total encountered LPS and that LSECs are responsible for the other 75% (Yao et al, 2016). Binding of PAMPs or DAMPs to toll-like receptors of LSECs result in the production of pro-inflammatory cytokines and thereby initiation of the innate immune response. However, LSECs also show increased tolerance to LPS upon re-exposure, this is thought to be a mechanism of LSECs in response to low levels of dietary LPS to prevent unnecessary inflammation and activation of the liver (DeLeve, 2015; Pandey et al, 2020). This is not via the downregulation of PRRs, but via the reduction of nuclear localization of nuclear factor-κB (NF-κB). Furthermore, LSECs show a reduction in the adhesion molecule CD54 upon LPS re-exposure that diminishes leukocyte adhesion and thereby reduces the level of inflammation (Uhrig et al, 2005).

When PAMPs or DAMPs trigger an inflammatory response by the liver, LSECs start to upregulate adhesion molecules for the extravasation of leukocytes to the site of inflammation. Leukocytes express cell surface receptors that allow them to bind to adhesion molecules onto the endothelium. In the post capillary venules, before the extravasation of leukocytes to the site of inflammation, leukocytes first roll along the vascular endothelium. Next, leukocytes start to express integrins, which allows for a strong binding between the leukocytes and endothelium. Finally, the leukocytes extravasate towards the site of inflammation within the liver, where they can execute their role in clearance of the insult. In non-LSEC endothelial cells, leukocyte rolling is mediated by selectins, however due to the low flow velocity in the endothelium controlled by LSECs, this rolling is not essential, which makes the start of the leukocyte extravasation process selectin-independent (Shetty et al, 2018). Another important immune related receptor is the FcγRIIb [fig-3]. This IgG Fc receptor, also called CD32b, is involved in the clearance of IgG immune complexes from the blood thereby keeping their plasma concentration low and preventing disease-related inflammation that these complexes can cause. LSECs express around 90% of the total FcγRIIb in the liver, highlighting the importance of LSECs in the clearance of IgG immune complexes (Ganesan et al, 2012). The immune complexes are taken up via an endocytic pathway, after which they are degraded into lysosomes (Lovdal et al, 2000). The LSECs are not only important for tissue homeostasis by their involvement in important signaling between liver cells, but also on their own by their high permeability, endocytotic capacity and immunological functions. Their scavenger receptors and immunomodulatory receptors as for instance toll-like receptors, complement these functions and are crucial for LSEC mediated tissue homeostasis.



(Figure retrieved from Gracia-Sancho et al, 2021.)

Fig. 3 Ways in which LSECs maintain homeostasis in physiological conditions.

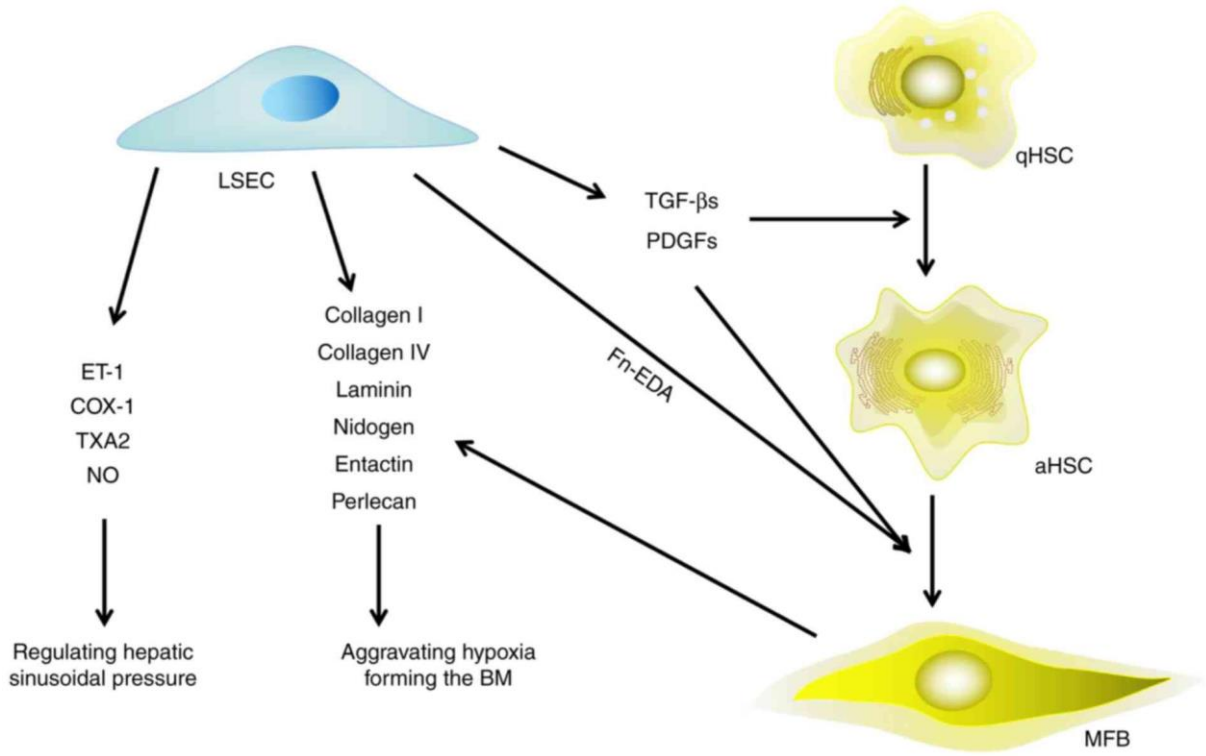
(1) LSECs characteristic morphology, lacking a basement membrane and having permeable fenestration, allows for the direct exchange of substances as oxygen and lipoproteins**. (2)** Via the production of the vasodilatory molecule NO, LSECs maintain HSCs quiescence and sinusoid vasodilation. Significant shear stress activates the transcription factor KLF2 that regulates the expression of eNOS and thereby the synthesis of NO. NO acts on a downstream signaling cascade in which sGC produces cGMP out of GTP resulting in vasodilation and plays a role in fatty acid β-oxidation in hepatocytes. **(3)** LSECs express a wide variety of receptors including the collagen-α-chain/mannose receptor as LYVE1, the hyaluronan/scavenger receptors as STAB1 and STAB2, the IgG Fc receptor FcγRIIb (CD32b), and VEGFR, important for maintaining homeostasis (Gracia-Sancho et al, 2021).

2.4. Changes in LSECs during hepatic fibrosis in CLDs

Hepatic fibrosis is a process that occurs when there is injury of the liver that cannot be resolved. When the liver comes across any type of injury, it will initiate a repair process that is characterized by an inflammatory response and limited amounts of collagen secretions in the liver microenvironment. When this response is successful it gets rid of the trigger responsible for the damage and the parenchymal cells regenerate and replace the damaged tissue. However, when the type of injury cannot be resolved, it will give rise to an ongoing repair response resulting in excessive amounts of ECM production by activated HSCs and other myofibroblast-like cells. Eventually the ongoing repair response fails and the damaged tissue does not regenerate but becomes replaced by the excessive amounts of ECM, secreted by cells part of the tissue microenvironment and other recruited ECM-depositing cells. These processes of tissue remodeling and scarring are well-known as being part of hepatic fibrosis. The tissue remodeling and scarring leads to loss of tissue integrity and eventually loss of tissue function (Bataller & Brenner, 2005; Lafoz et al, 2020). Historically, it was thought that hepatic fibrosis was irreversible. However, hepatic fibrosis has proven to be reversible upon removing the underlying etiology. This has even shown to be the case for advanced hepatic fibrosis. Initial processes of reversal of hepatic fibrosis are the loss of myofibroblast cells via senescence and apoptosis, loss of fibrous scars, a decrease in cytokine levels and an increase in collagenase activity.

However, removal of the underlying etiology is not possible for most of the CLDs. In these CLDs hepatic fibrosis is established as being irreversible (Aydın & Akçalı, 2018).

LSECs play a role in hepatic fibrosis in three different ways. One of them has already been mentioned, which is the regulation of vascular tone via the release of vasodilators and vasoconstrictors. Another way in which LSECs influence the fibrotic process is by the direct secretion of ECM components. These secretions involve proteins such as laminin, collagen IV and nidogen that can form a basement membrane leading to a decrease in the exchange of substances across the endothelium. Furthermore, the ECM components result in progression of hepatic fibrosis by contributing to tissue stiffness and by the replacement of damaged tissue. Lastly, LSECs contribute to fibrosis indirectly by the secretion of proinflammatory and profibrotic factors. Two of these factors are transforming growth factor beta (TGF-β1) and platelet-derived growth factor (PDGF). They both play a role in the activation and migration of HSCs. Moreover, they promote the transdifferentiation of HSCs into myofibroblasts, with TGF-β1 often being referred to as a master regulator of this process (Cheng et al, 2021). [fig-4]. TGF-β1 is also known as an inducer of endothelial or epithelial mesenchymal transfer in fibrotic diseases in endothelial cells, tissue-resident fibroblasts, epithelial cells, pericytes and CD34+ progenitor cells (Pardali et al, 2017). However, there is lack of unambiguous in vivo evidence of endothelial or epithelial mesenchymal transition in hepatic fibrosis. In combination with PDGF signaling, TGF-β signaling is considered the main fibrogenic pathway that promotes HSC activation and drives the production of the excessive amounts of ECM in hepatic fibrosis (Dewidar et al, 2019). Furthermore, TGF-β recruits immune cells to the site of inflammation (Caja et al, 2018). PDGF is a ligand for the PDGF receptor (PDGFR), which is mainly expressed on mesenchymal cells. In hepatic fibrosis, production of PDGF is upregulated by KCs, recruited macrophages and LSECs. Moreover, PDGFR is upregulated by myofibroblasts. Activation of PDGFR stimulates mitogenic, motogenic and chemoattractant effects in mesenchymal cells, stimulating the proliferation, chemotaxis and recruitment of myofibroblast cells in hepatic fibrosis as HSCs and PMFs (Klinkhammer et al, 2018). Lastly, in hepatic fibrosis, TGF-β1 and PDGF disrupt the balance between matrix metalloproteinases and their inhibitors. They shift it towards tissue inhibitors of matrix metalloproteinases (TIMPs) by their upregulation. The TIMPs inhibit matrix metalloproteinases (MMPs), which function as collagenases. In this way TIMPs prevent MMPs from degrading the excess of collagen secreted by activated HSCs stimulated by LSEC and non-LSEC produced TGF-β and PDGF, resulting in further progression of fibrosis. Balance between TIMPs and MMPs is essential for tissue homeostasis and prevention of hepatic fibrosis (Roeb, 2018).



(Figure retrieved from Cheng et al, 2021)

Fig. 4 Three different processes in which LSECs contribute to hepatic fibrosis

LSECs contribute to hepatic fibrosis **(1)** by regulating the balance between vasoconstrictors and vasodilators thereby regulating vascular tone, **(2)** by the direct secretion of ECM forming a basement membrane and **(3)** by the secretion of growth factors as TGF-β and PDGF that activate HSCs and stimulate HSC transdifferentiation to myofibroblasts. MFB, myofibroblast; qHSC, quiescent HSC; aHSC, activated HSC; COX-1, cyclooxygenase 1; TXA2, thromboxane A2; ET-1, endothelin 1; NO, nitric oxide; Fn-EDA, fibronectin-splice variant containing extra domain A; BM, basement membrane (Cheng et al, 2021).

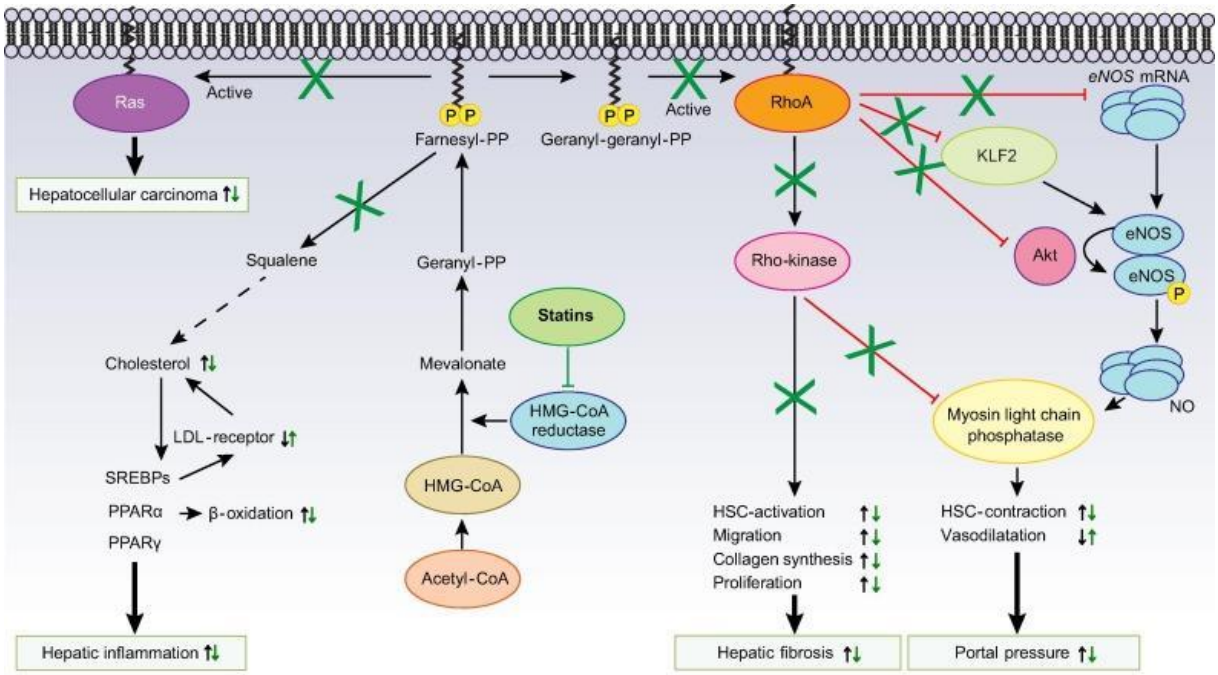
3. Attenuation and reversal of hepatic fibrosis by normalization of LSEC function

Some of the most important functions and characteristics of LSECs have been highlighted, in normal tissue homeostasis and in fibrosis. This section will focus on therapeutics that are able to restore LSEC function and thereby stimulate them to carry out their important roles in maintaining tissue homeostasis. In this way LSECs have shown to be able to attenuate and reverse hepatic fibrosis, but also CLDs. This section will provide an overview of therapeutics that were able to achieve attenuation and reversal of hepatic fibrosis targeting LSECs, starting off with statins.

3.1. Statins interfering with NO production of LSECs

Statins have been under investigation for treatment of CLDs for the past years. They were already in use as the main lipid lowering treatment for dyslipidemia. Moreover, they have a beneficial role in the primary and secondary prevention of cardiovascular diseases (Zhai et al, 2020). Over the past decade statins have shown beneficial effects in the treatment of advanced CLDs. The effects include: a reduced portal pressure, improvement of LSEC and HSC dysfunction and decreased fibrogenesis (Bosch et al, 2020). Statins induce the transcription factor KLF2 by inhibition of the RhoA/Rho-kinase pathways. The inhibition of this pathway results in a decrease of small GTPases that influence signaling pathways of LSECs and HSCs. It leads to induction of KLF2 and other molecules involved in

the production of NO. This increase in NO results in a lower HSC contractility and vasodilation resulting in a lower portal pressure and attenuation of reversal of CLD. Moreover, the inhibition of Rho-kinases prevents HSC activation, migration, collagen synthesis and proliferation, contributing to attenuation of hepatic fibrosis [fig-5] (Pose et al, 2019; Abraldes et al, 2007).



(Figure retrieved from Pose et al, 2019).

Fig. 5 Pleiotropic effects of statins in the liver.

Statins decrease the activation of small GTPases in the liver such as RhoA and Ras in LSECs and HSCs. The decrease in activation of RhoA results in changes in subsequent signaling pathways that lower portal pressure and cause less fibrinogenesis. The decrease in small GTPases as well lower hepatic inflammation and might prevent the development of hepatocellular carcinoma by acting on Ras, which is involved in growth and proliferation signals (Pose et al, 2019).

Furthermore, upregulation of KLF2 using a statin called simvastatin has shown that in a coculture of HSCs and LSECs, simvastatin attenuates LSEC dysfunction by improvement of HSC phenotype. The attenuation was caused by VEGF produced by the HSCs. Moreover, cirrhotic rats treated with simvastatin showed upregulation of KLF2, the transition of activated HSCs to quiescent HSCs and a noticeable reduction in hepatic fibrosis. The rats also showed a reduction in portal pressure and a significant improvement in the dysfunctional liver endothelium (Marrone et al, 2014). Another remarkable study regarding statins, is a study that evaluates statin treatment across CLD patients. This study selected 234 patients for statin treatment. Each separate patient had 2 control patients selected based on age, sex, CLD and body mass index. It was found that the patients treated with statins had significantly less stiff liver tissue compared to patients that did not receive any statins. Moreover, the statin-group had a reduced inflammatory activity and reduced markers for portal hypertension. Furthermore, the statin-group was found to be less likely to develop a decompensated liver (Markova et al, 2020). This result is promising since people that have developed a decompensated liver have a high mortality rate and are in need of intensive care (Mansour & McPherson, 2018). Lastly, a study that observed the use of statins in chronic HCV patients with advanced fibrosis showed that in the statin-group progression of fibrosis occurred in 3/29 patients (10%) and for non-statin group in 145/514 patients (29%). Over the 3.5 year running of this study,

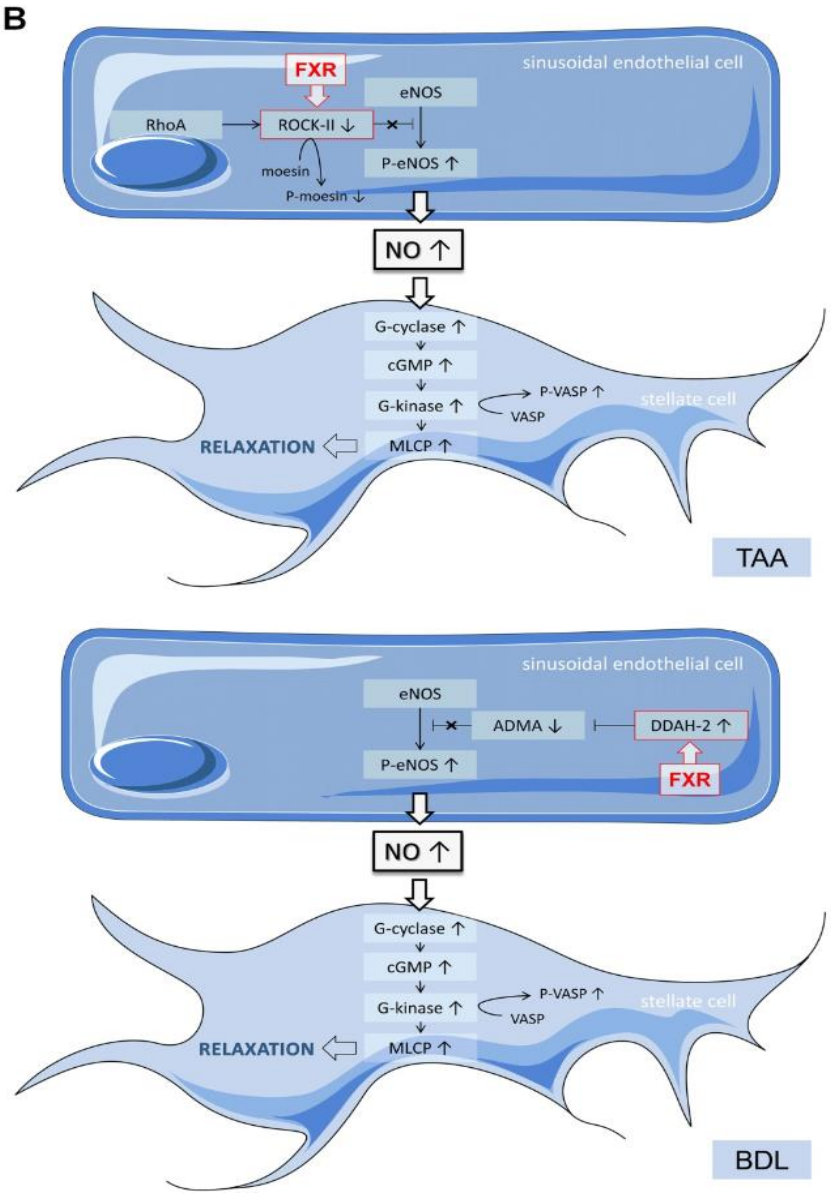
both groups were examined for the progression of fibrosis based on the Ishak fibrosis scale. Interestingly, over the 3.5 years, the statin-group scored a mean change of negative 0.32 indicating attenuation of hepatic fibrosis. The non-statin group scored positive 0.42 indicating progression of fibrosis (Simon et al, 2015). All in all, statin treatment is associated with attenuation of hepatic fibrosis in CLDs. This attenuation is mediated by LSECs and HSCs via inhibition of the Rho/Rho-kinase pathway. Statins have been questioned in their use in CLDs because of their hepatotoxicity, however more and more studies are confirming their safety in treatment of CLDS (Vargas et al, 2017). The future prospect of statins in CLDs is promising and we might encounter their use as a regular treatment in CLDs, possibly combined with other drugs that help statins in the treatment of CLDs.

3.2 Nuclear receptor agonists and their effects on endothelial dysfunction

3.2.1 The farnesoid X receptor

LSECs contain a subset of different nuclear receptors that have shown to play a role in fibrosis related LSEC injury. One of them is the farnesoid X receptor (FXR) (Ma et al, 2020). The FXR is involved in the metabolism of bile acids, glucose, lipids and amino acids (Han, 2018). The FXR is highly expressed in the liver and small intestine. In the liver, the FXR is expressed by LSECs, HSCs, KCs and hepatocytes (Jiao et al, 2014; Jin et al, 2020; Alawad et al, 2016; Verbeke et al, 2014). In all of these cells, FXR agonists have shown beneficial outcomes towards liver functioning and the attenuation of CLDs. In hepatocytes, FXR agonists have shown to suppress de novo lipogenesis and promote the oxidation and clearance of triglycerides, reducing hepatic steatosis and hyperlipidemia (Jiao et al, 2014). Moreover, in KCs, FXR agonists have shown to protect the liver from the proinflammatory response during ischemia/reperfusion injury (Jin et al, 2020). Furthermore, in HSCs, treatment with FXR agonists resulted in reduced expression of collagen type I α chains, TGF-β and smooth muscle actin, which is a marker of HSC phenotype to myofibroblast transition. Lastly, in animal studies, the FXR agonist obeticholic acid prevented development of hepatic fibrosis. (Alawad et al, 2016). It would be very interesting to go into further detail about how the FXR agonists work in hepatocytes, KCs and HSCs, however that is beyond the scope of this thesis. Continuing with the LSECs, a study showed that in TAA-induced cirrhotic rats and BDL-induced cirrhotic rats, obeticholic acid reduced portal pressure via a vasodilatory mechanism. However, the vasodilatory mechanism was not the same in both fibrotic rat models, indicating that the underlying etiology of the induced disease influences the efficacy of treatment with FXR agonists. In both models there was a significant increase in Rho-kinase activity, as seen by the increased phosphorylation of the Rho-kinase substrate moesin, resulting in less hepatic fibrogenesis and a lower portal pressure. TAA-induced cirrhotic rats treated with FXR agonist INT-747 showed downregulation of the RhoA/Rho-kinase pathway signaling, eventually resulting in higher levels of NO production by LSECs, causing vasodilatory effects in HSCs [fig-6] (Verbeke et al, 2014). Active forms of Rho and ROCK-II, another important protein in this pathway, both decrease eNOS phosphorylation and eNOS mRNA stability, causing downregulation of eNOS expression and activity, resulting in less LSEC NO production (Rikitake et al, 2005). As indicated in the section above, this pathway is also affected by statins. BDL-induced cirrhotic rats treated with INT-747 showed upregulation of dimethylaminohydrolase (DDAH), which is an enzyme that prevents accumulation of methylarginases that inhibit eNOS activation. This results in a higher production of NO, causing vasodilatory effects in HSCs [fig-6] (Verbeke et al, 2014). A more recent study investigated the effects of the novel FXR agonist PX20606 in a fibrotic animal model. They found that treatment with PX resulted in a significantly reduced portal pressure on short term treatment by restoring endothelial function. Long term treatment inhibited sinusoidal vascular remodeling and decreased portal pressure even further. Moreover, they found a significant decrease of 43% in the fibrotic area (Sirius red stain) of CCL4-induced fibrotic mice treated with PX compared to their

controls. This reduction in fibrotic area was 22% when the mice were treated with obeticholic acid. Furthermore, it was found that PX also decreased the expression of collagen type I α chains, TGF-β and smooth muscle actin, just like obeticholic acid. In rat and human derived LSECs, PX caused upregulation of eNOS and DDAH that both lead to increased levels of LSEC NO production, maintaining LSEC phenotype. Interestingly, PX and obeticholic acid caused upregulation of DDAH isoforms in human umbilical vein endothelial cells (HUVECs) and this upregulation was even higher in LSECs. The same holds true for the increase of eNOS in HUVECs and LSECs after FXR agonist treatment, indicating that LSECs are more sensitive to FXR-mediated eNOS expression (Schwabl et al, 2017). Clinical trials have to rule out their effects in patients with other CLDs. Interestingly since 2016, obeticholic acid has had provisional approval in the United States to be used in the treatment of primary biliary cholangitis. Within a year, the treatment had caused several adverse events that resulted in the release of a warning letter from the FDA. These adverse events were characterized by jaundice, ascites, decompensated liver eventually causing liver failure. Therefore, when starting treatment, patients are monitored to test the efficacy and safety for each individual patient. When adverse events are detected, treatment should immediately be stopped. This experience should be considered when starting clinical trials for FXR agonists in other CLDs. Currently, it is not known what causes these side-effects. As indicated earlier, the FXR agonists have been beneficial in improving liver function and the attenuation of CLDs in many cell types. This might be one of the reasons why the side-effects are thought to be dose related. For instance jaundice, which can indicate a worsened hepatocyte function and a lowered bile processing, might be caused by dose-related side-effects of an FXR agonist that was intended to induce beneficial effects via the HSC FXR, but also caused dose-related side-effects due to for instance overstimulation of the hepatocyte FXR (LiverTox, 2019). FXR agonists have shown to improve endothelial dysfunction, hepatic fibrosis and portal hypertension in fibrotic animal models. However, clinical trials still have to rule out their safety and efficacy in treatment of hepatic fibrosis across other CLDs.



(Figure retrieved from Verbeke et al, 2014)

Fig. 6 Vasodilatory mechanism targeted by the FXR agonist INT-747 depends on the underlying etiology of cirrhosis.

The FXR agonist INT-747 can cause vasodilation in HSCs via the upregulation of LSEC NO production. Depending on the underlying etiology of the induced cirrhosis being TAA-induced or BDL-induced, the vasodilatory mechanism is different. In TAA-induced cirrhotic rats, INT-747 downregulates the RhoA/Rho-kinase signaling pathway, by downregulating ROCK-II. This results in higher expression and activity of LSEC eNOS and therefore higher levels of LSEC NO production that induces vasodilatory effects in HSCs. In BDL-induced cirrhotic rats, INT-747 promotes upregulation of DDAH, causing the inhibition of asymmetric dimethylarginine (ADMA) that are able to inhibit eNOS. This results in more LSEC NO production that induces vasodilatory effects in HSCs (Verbeke et al, 2014).

3.2.2 The peroxisome proliferation-activated receptor

Another family of nuclear receptors involved in fibrosis-related LSEC injury is the peroxisome proliferation-activated receptor (PPAR) family. There are 3 different PPARs; PPARα, PPARγ, and PPARβ/δ. PPARα is the highest expressed PPAR in the liver. In a TAA-induced cirrhotic rat model, LSECs were most abundant in expressing PPARδ, however during cirrhosis LSEC PPARα expression was increased, which in normal physiological conditions was mostly expressed by hepatocytes. PPARγ was predominantly expressed in KCs and was upregulated in HSCs during cirrhosis. The TAA-induced cirrhotic rat model closely resembles the deregulation in PPAR expression levels in human cirrhosis (Boyer-Diaz et al, 2020). PPARs are involved in many different functions related to metabolism as bile acid, glucose and lipid metabolism, but also in regeneration, differentiation, proliferation and inflammatory reactions (Peyrou et al, 2012). Dysregulation of the PPARs expression or activity has been associated with progression of liver disease, insulin resistance and fibrosis (Boyer-Diaz et al, 2020). A study has shown that the PPARδ agonist KD3010 is able to attenuate and reverse liver fibrosis in a CLL4-induced fibrotic animal model. The group treated with KD3010 showed a decrease in fibrosis (Sirius red stain), a reduction in hydroxyproline (which can be used as measure for total collagen), decrease in smooth muscle actin, decrease in mRNA expression of collagen type I α chains and reduction of TIMPs (Iwaisako et al, 2012). Hydroxyproline is as well a component of elastin, however total elastin is usually measured by the protein desmosine (Stoilov et al, 2018). Another study regarding an agonist of PPAR, lanifibranor, has shown to significantly improve LSEC phenotype in cirrhotic rat models. Cirrhotic rats treated with lanifibranor showed a significant increase of 25% in LSEC fenestrae compared to their controls, indicating reversion of the capillarization process. Moreover, the treated group showed reduced levels of liver von-Willebrand factor, which is normally upregulated when there is any type of endothelial damage. Lastly, LSECs of the treated group showed a significant reduction in mRNA levels of adhesion molecules compared to their controls, suggesting normalization of LSECs. All in all, this study showed that lanifibranor was able to reduce portal pressure, protect LSEC and HSC phenotype, reduce hepatic inflammation and significantly regress hepatic fibrosis by 32% in a cirrhotic rat model. Lanifibranor reduces portal hypertension and hepatic fibrosis (Boyer-Diaz et al, 2020). Lanifibrandor is currently in phase 2 of clinical trials for treatment of non-alcoholic steatohepatitis (a form of NAFLD), along with some other PPAR agonists for the treatment of CLDs. However, the number of clinical trials of PPAR agonists related to treatment of CLDs is still low (Cheng et al, 2019). In pre-clinical models PPAR agonists show great results on normalization of LSEC phenotype thereby attenuating and reversing hepatic fibrosis. Now, clinical trials should further elucidate this potency shown in pre-clinical models to eventually make PPARs agonist a potent treatment for hepatic fibrosis in CLDs.

3.2.3 The liver X receptor

The last nuclear receptor to discuss regarding LSEC-mediated liver injury is the liver X receptor (LXR). The LXR is involved in the metabolism of cholesterol via its binding to its endogenous ligands; oxysterols (oxidized cholesterol). LXRs also have shown a role in lipid and carbohydrate metabolism, cellular differentiation, apoptosis and immune responses. The LXRs consist of the LXRα and LXRβ receptor. LXRα is expressed in several tissues, with the highest expression in the liver (Patel et al, 2008). The LXR is expressed by hepatocytes, KCs, HSCs and LSECs, but also by leukocytes (Endo-Umeda & Makishima, 2019). LXR forms a heterodimer with the retinoid X receptor (RXR) upon binding of an LXR specific ligand. This results in a conformational change that can lead to transcription of target genes but can also cause inhibition of certain genes via trans-repression (Patel et al, 2008). In CCL4-induced fibrotic mice models, LXRα is upregulated in LSECs. When LXRα was deleted, it resulted in exacerbated lesions in the fibrotic mice model, as well as worsened

inflammation and increased collagen deposition. Moreover, it aggravated the capillarization of LSECs. Furthermore, deletion of LXRα resulted in increased hedgehog-signaling by LSECs, which is as well the case during LSEC capillarization. (Xing et al, 2016). Increased hedgehog-signaling in liver disease has shown to promote hepatic fibrosis and liver cancer (Gao et al, 2018). Normally, LSECs show capillarization after around two days of in vitro culturing. However, when culturing LSECs with the LXR agonist T0901317, LSECs showed inhibition of capillarization after 2 days. T0 interferes with the mRNA levels of hedgehog ligand (Shh), the cell surface receptor of hedgehog ligand (patched1) and the hedgehog target gene Gli2. T0 showed to inhibit the mRNA levels of all of these hedgehog signaling pathway components three days after culturing. Moreover, when LSECS were cultured with T0 and the hedgehog signaling agonist SAG, downregulation of markers for capillarization caused by T0 was blocked, indicating capillarization is mediated via hedgehog signaling (Xing et al, 2016). Another study showed that hedgehog agonists promoted upregulation of capillarization-associated genes. Moreover, it was found that LSECs isolated from transgenic mice with knockdown of smoothened, which is a G-protein coupled receptor essential for the hedgehog signaling pathway, caused inhibition of hedgehog signaling in transgenic mice, resulting in downregulation of capillarization-associated genes and retention of the LSEC fenestrations (Xie et al, 2013). Furthermore, in HSCs, inhibition of hedgehog signaling lead to decreased levels of smooth muscle actin and decreased secretion of collagen type I (Li et al, 2015). These findings suggest that LXRα signaling prevents capillarization and protects LSEC phenotype via inhibition of hedgehog signaling, which might contribute to the attenuation and reversal of hepatic fibrosis. A recent study regarding a reverse agonist of LXRα found that SR9243 was able to significantly decrease fibrosis in CCl4-induced fibrotic mice and in BDL-induced fibrotic mice. As opposed to agonists that initiate receptor activity, inverse agonists stabilize active receptors and reduce their activity. Agonists and reverse agonists both have intrinsic activity, however this is not the case for antagonists. Antagonists prevent agonists and reverse agonists from binding and activation. In this way antagonists so to speak switch off the receptor (Khilnani & Khilnani, 2011). Mice treated with the reverse agonist SR9243 had significantly lower levels of hydroxyproline and collagen type I α chains compared to their controls. This was true for both differently induced animal models. SR9243 was able to inhibit CLL4- and BDL-induced hepatic fibrosis in mice. This indicates that LXR targeting has a high potential for becoming an anti-fibrotic therapeutic. Further studies are needed to elucidate the biosafety of SR2943, however SR9243 showed no hepatotoxicity in both models, which had been a downside of earlier used LXR agonists (Huang et al, 2018).

4. LSECs now and future perspectives

This thesis has highlighted the most important functions of LSECs in normal liver homeostasis. These functions exist of regulating the exchange of molecules and foreign substances back and forth between the portal blood, space of Disse and hepatocytes. LSECs make this possible because of their remarkable morphological characteristics and capabilities as being highly permeable because of their fenestrae and the lack of basement membrane. Furthermore, they have the highest endocytotic capacity due to their many scavenger receptors. Not to forget their immunological receptors as the toll-like receptors and the FcγRIIb that play a role in the initiation and stimulation of immune reactions and help to clear immune complexes. Secondly, LSECs are important in keeping a low vascular tone in the liver vasculature, thereby promoting a high exchange of molecules across the endothelium. They do this by keeping a balance between vasoconstrictors as endothelin-1 and vasodilators as NO. With NO being especially important in maintaining LSEC phenotype and preventing capillarization and dysfunction. Lastly, LSECs keep HSCs in a quiescent state in normal liver homeostasis via a VEGF-dependent signaling between the two.

Continuing, in hepatic fibrosis LSECs have shown to lose their characteristic phenotype and regulatory functions. The loss of LSEC phenotype has been identified as an initial trigger for hepatic fibrosis and results in the loss of controlling HSCs quiescence. In hepatic fibrosis, LSECs shift from the characteristic LSEC phenotype, in which they lack the basement membrane and have numerous fenestrae, into a vascular endothelial-like phenotype with little to no fenestrae and the formation of a basement membrane in a process called capillarization. This results (1) into a disbalance between the vasoconstrictors and vasodilators, creating a contractile environment, thereby negatively influencing portal pressure and fibrosis, (2) in stimulation of the direct production of ECM proteins, contributing to tissue stiffness and progression of fibrosis, and (3) in secretion of growth factors as TGF-β1 and PDGF, activating and driving HSCs to produce an excess of collagen, promoting transition of HSC to myofibroblast, recruiting of immune cells and deregulating the balance between MMPs and TIMPs.

Furthermore, normalization of LSEC phenotype via a subset of different therapeutics has shown to significantly reduce hepatic fibrosis in multiple CLDs in vitro and in vivo. Compounds such as statins targeting the RhoA/Rho-kinase pathway thereby promoting NO production or compounds that function as agonists of nuclear receptors as the FXR, PPAR or LXR that can normalize LSEC phenotype, ameliorate endothelial dysfunction and attenuate hepatic fibrosis. Based on these findings there can be concluded that normalization of LSEC function definitely has a role in the attenuation and reversal of fibrosis. Normalization of LSECs function is one of the promising ways to stop and possibly reverse hepatic fibrosis.

Over the past few years LSECs have started to get more attention regarding their role in hepatic fibrosis and CLDs. This might be one of the reasons for the fact that there are still few clinical trials of therapeutics that aim to normalize LSEC function to tackle hepatic fibrosis in CLDs. For the past two decades antifibrotic treatment has mainly focused on apoptosis or deactivation of HSCs, stimulation of ECM degradation, inhibition of myofibroblast activation, the usage of liver-protective agents reducing damage and treatment of the underlying etiology. The HSCs and PMFs have always been the main target in the search for antifibrotic therapeutics and still are. The research history of these two cells in hepatic fibrosis is already multiple decades long with until now still no antifibrotic treatment for hepatic fibrosis on the market yet. However, the research history of LSECs is still in an early phase, with many secrets to be uncovered. The impact of dysfunctional LSECs in the fibrotic process has been neglected for quite a while now and hopefully, with all the recent attention that they got and potential that they showed, they will become more important in the search for antifibrotic treatment in CLDs. The answer to antifibrotic therapies might not lie within the results of a single therapeutic agent. Hepatic fibrosis is a complex process that has a multicellular origin. Not one cell type is the key player in hepatic fibrosis, but it is the interplay and disbalance between HSCs, LSECs, KCs, hepatocytes, other tissue-resident cells, and immune cells. Combination therapy of multiple antifibrotic agents might be the best possible way to attenuate and reverse hepatic fibrosis. Normalization of LSEC function has proven its potential in the attenuation and reversal of hepatic fibrosis. It is now only a matter of time and willingness of the research community to get the LSEC targeted treatment out there and contribute to the relieve of the high burden that CLDs and hepatic fibrosis have on society. LSECs are basically ‘screaming’ for attention and hopefully they will be heard by the research community, giving rise to a switch in the research field from the intensively studied HSCs and PMFs towards the neglected LSECs.

5. Illustrative overview of this thesis

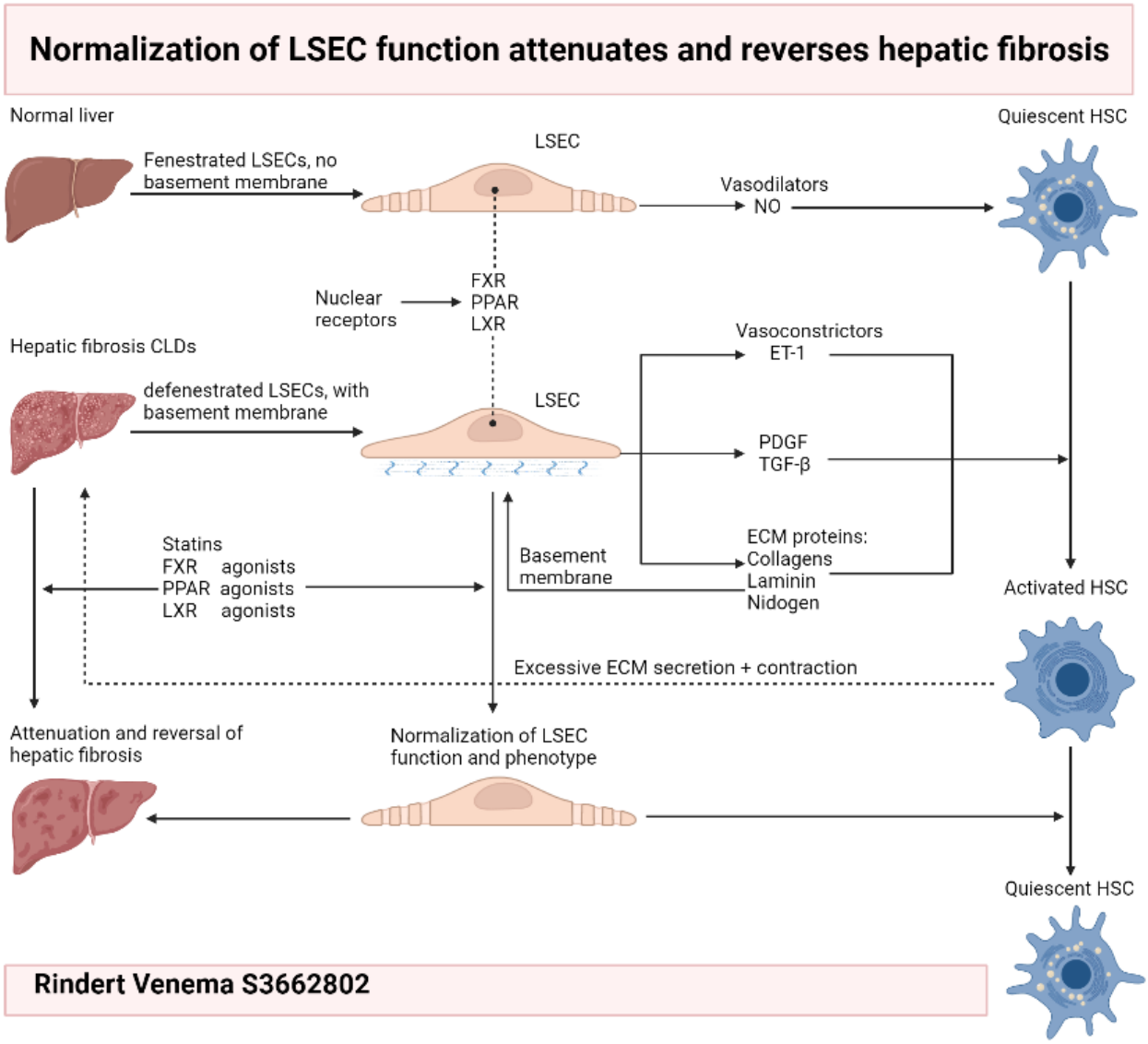


Fig. 7 Overview of this thesis. (Created in BioRender.com)

6. References

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