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ROLE OF PLATELET-DERIVED GROWTH FACTOR B IN CHOLESTEROL AND GLUCOSE METABOLISM

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THESIS FOR DOCTORAL DEGREE (Ph.D.)

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ABSTRACT

Platelet-derived growth factor (PDGFB) is a critical molecule for recruiting pericytes. Due to the fundamental role of the pericytes in angiogenesis and vascular stability, PDGFB knockout mice die perinatally from severe hemorrhage and edema. PDGFB retention motif knockout mouse (*Pdgfb*$^{ret/ret}$) is one of the available models to study the postnatal effect of PDGFB signaling. My PhD projects intensively used *Pdgfb*$^{ret/ret}$ to explore the role of PDGFB in cholesterol and glucose metabolism.

In the brain, the pericyte coverage on the blood-brain barrier (BBB) of *Pdgfb*$^{ret/ret}$ is reduced by 70%, which leads to increased permeability to a series of high- and low-mass tracers. Physiologically cholesterol metabolism in the brain is isolated from the other organs or the circulation by BBB. **Paper I** and **II** used *Pdgfb*$^{ret/ret}$ mice as a BBB leakage model and studied the metabolic changes of cholesterol metabolism in the brain when BBB lose its integrity. Our results show that in *Pdgfb*$^{ret/ret}$, more plant sterols are accumulated in the brain due to increased influx. Meanwhile due to increased efflux of 24(S)-hydroxycholesterol, the negative feedbacks to *de novo* biosynthesis of cholesterol in the brain is diminished.

Liver has been considered as a special organ for PDGFB signaling, because recruitment of the hepatic stellate cells (HSCs), also disputably known as the liver pericytes, are recruited independently of PDGFB. PDGFB is known to be expressed from the endothelial cells of portal veins, but its physiological role is still poorly investigated. In **Paper III**, we describe for the first time that cholangiocytes, the epithelial cells of bile ducts, are also expressing PDGFB. Similar to pericyte deficiency on BBB, *Pdgfb*$^{ret/ret}$ mice show deficiency of periductal mesenchymal cells, together with bile duct dilation and portal inflammatory infiltrate (PII). PII is of great clinical importance, because it is an early marker ubiquitously present in many biliary and non-biliary liver diseases. Using *Pdgfb*$^{ret/ret}$, together with PDGFB knockouts and *Sox2*-Cre;*PDGFRβ*$^{+/D849V}$ mutants in which PDGFRβ is over-active, we confirmed that elevated PDGFB signaling leads to PII directly. In *Pdgfb*$^{ret/ret}$, PII is accompanied by a lopsided immune profile, with increased CD8 T cells and decreased NKT cells, which mimics the immune profile at the steatosis stage of non-alchoholic fatty liver diseases. From metabolic perspective, we show that immune response in *Pdgfb*$^{ret/ret}$ leads further to intolerance to high cholesterol diet and susceptibility to hepatic steatosis by suppressing VLDL secretion.

Paper IV focuses on the function of PDGFB in pancreatic islets. We show that PDGFB is not a key factor to maintain the pericyte coverage in pancreatic islets. In *Pdgfb*$^{ret/ret}$, the slightly disrupted PDGFB signaling is not enough to affect vascular functions in pancreatic islets. The first phase of insulin secretion in *Pdgfb*$^{ret/ret}$ is earlier and stronger, and our current data indicates a systematic factor as the underlying mechanism.

LIST OF SCIENTIFIC PAPERST

This thesis is based on the publications listed below

I. **Effects of a disrupted blood-brain barrier on cholesterol homeostasis in the brain**
Saeed, AA; Genové, G; **LI, T**; Lütjohann, D; Olin, M ; Mast, N; Pikuleva, IA; Crick, P; Wang, Y; Griffiths, W; Betsholtz, C; Björkhem, I
J Biol Chem, 2014, 289(34), 23712-22

II. **Increased flux of the plant sterols campesterol and sitosterol across a disrupted blood-brain barrier**
Saeed, AA; Genové, G; **LI, T**; Hülshorst, F; Betsholtz, C; Björkhem, I; Lütjohann, D
Steroids, 2015, 99(Pt B), 183-8

III. **Role of platelet-derived growth factor B in liver pathophysiological cholesterol regulation**
LI, T; Saeed, AA; Saxena, A; Hong, JW; Lavergne, M; Schieb, B; Spahn, C; Mäe, MA; Mössinger, C; Kuiper, RV; Olson, LE; Betsholtz, C; Björkhem, I; Poljakovic, M; Genové G
Submitted manuscript

IV. **Role of platelet-derived growth factor B in insulin secretion**
LI, T; Ning, Ch; Hong, JW; Lavergne, M; Nyqvist, D; Genové, G
Manuscript

Additional publications not discussed within this thesis

Role of tumor pericytes in the recruitment of myeloid-derived suppressor cells
Hong, JW; Tobin, NP; Rundqvist, H; **Li, T**; Lavergne, M; García - Ibáñez, Y; Qin, HY; Paulsson, J; Zeitelhofer, M; Adzemovic, MZ; Nilsson, I; Roswall, P; Hartman, J; Johnson, RS; Östman, A; Bergh, J; Poljakovic, M; Genové, G
Journal Of The National Cancer Institute, 2015, Vol. 107(10)

CONTENTS

LIST OF ABBREVIATIONS

ABCA	ATP-binding cassette transporter sub-family A
ABCB	ATP-binding cassette sub-family B
ABCG	ATP-binding cassette sub-family G
ApoA1	Apolipoprotein A1
ApoB	Apolipoprotein B
BBB	Blood-brain barrier
CNS	Central Nervous System
CYP7A	Cholesterol 7alpha-hydroxylase
CYP27A	Sterol 27-hydroxylase
CYP46A1	Sterol 24-hydroxylase
DAG	Diacylglycerol
ERK	Extracellular signal-regulated kinases
FGF	Fibroblast growth factor
GLUT4	Glucose transport type 4
GSK	Glycogen synthase kinase
HCD	High cholesterol diet
HDL	High-density lipoprotein
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzym A
HSCs	Hepatic stellate cells
HSPG-BD	Heparin sulfate proteoglycan binding domain
JNKs	c-Jun N-terminal kinase
IRS	Insulin receptor substrate
K_{ATP} channel	ATP-sensitive potassium channel
LDL	Low density lipoprotein
MTTP	Microsomal triglyceride transfer protein
ORO	Oil-Red O
PDGFB	Platelet-derived growth factor B
PDGFR	Platelet-derived growth factor receptor
Pdgfb$^{ret/ret}$	PDGFB retention motif knockout
PKB	protein kinase B
ROS	Reactive oxygen species
SREBP-1	Sterol regulatory element-binding protein 1
StARD1	Steroidogenic acute regulatory
TG	Triglyceride
TGF-β	Transforming Growth Factor β
TNF-α	Tumor Necrosis Factor α
VLDL	Very low density lipoprotein

1 INTRODUCTION

1.1 CHOLESTEROL PHYSIOLOGY

Cholesterol ($C_{27}H_{46}O$) is a lipid molecule essential for cell membranes of all animals. In adult humans, the average turnover of cholesterol is estimated to be 1.2g per day. Structurally, cholesterol has a steroid ring structure at the centre with a polar hydroxyl group on one side and a nonpolar hydrocarbon tail on the other side. When forming cell membranes, the hydrocarbon tail is sitting in the hydrophobic plane with the hydroxyl head facing either out or into the cell. The hydroxyl group interacts with polar head groups of different phospholipids and sphingolipids, thus forming the lipid bilayer. Cholesterol is of great importance for the structural integrity of the cell membrane. The cholesterol content and distribution in cell membranes varies in different cell types, and cholesterol can modulate these membranes' physical characteristics. Cholesterol-rich regions have higher fluidity because cholesterol molecules are inserted between acyl chains, thus preventing them from crystallization and limiting their movements. Meanwhile, cholesterol increases the bilayer's thickness, thus decreasing the permeability to soluble ions and small molecules[1].

However, cholesterol is not only a structural component of cell membranes, but also plays an important role in cell signalling. Caveolin is a cholesterol-binding protein highly concentrated on a small dynamic lipid patch on cell plasma membranes called caveolae. Studies have shown that many membrane receptors are located on caveolae or caveolae-like domains, where caveolin functions as a bridge between the receptor and its effector to transduce signals[1]. Besides, caveolae itself is a cholesterol-rich region, and it has been shown that cholesterol amount also affect the receptors' stability by modulating the physical characteristics of caveolae[2]. Meanwhile cholesterol is the precursor of many steroid hormones in the body such as glucocorticoids, sex hormones and vitamin D. Steroid hormones are lipid-soluble, so they can easily diffuse via cell membrane and bind to their receptors in the cytoplasm. Then the activated receptor can be translocated into the nucleus and cast its effect by binding to genomic DNA and regulating its transcription. Through this ligand-receptor mechanism, steroid hormones are involved in different biological process, such as metabolism, immune response and development of sexual characteristics[3].

1.1.1 Cholesterol homeostasis in liver

Liver is the key organ to maintain cholesterol homeostasis outside the brain. Through blood circulation, the liver distributes necessary cholesterol to the peripheral tissues and recycles the

surplus. Through enterohepatic circulation, the liver intakes cholesterol from dietary and excrete excessive cholesterol into the intestine in the form of bile. Meanwhile, the liver compensates 20-50% of all cholesterol in mammals through *de novo* biosynthesis and this process is precisely regulated by the rate-limiting enzyme 3-hydroxy-3-methylglutaryl-coenzym A (HMG-CoA) reductase[4]. In this thesis, the *de novo* biosynthesis will not be further introduced, since we did not detect major changes in this process when exploring the role of PDGFB signalling in liver.

1.1.1.1 Liver anatomy

From metabolic perspective, the basic functional unit in the liver is the hepatic acinus, which is formed by two portal triads, two central veins and the tissue in-between. A portal triad consists of a hepatic artery, which supplies the liver with oxygenated blood, a hepatic portal vein, which brings nutrient-rich blood directly from the intestine, and a bile duct, which excretes excessive cholesterol and bile products out of the body. Blood converged from both the hepatic portal vein and the hepatic artery drains into the central vein through a highly-permeable capillary called sinusoid. As shown in Figure 1, sinusoid is composed of endothelial cells and aligns closely with the bile ducts. Lining the sinusoid there are kupffer cells, the specialized macrophages and hepatic stellate cells (HSCs), the liver pericytes which are critically involved in the pathogenesis of hepatic fibrosis. Around sinusoids, 70-85% of the liver mass is filled with hepatocytes, the major cell type of hepatic parenchymal tissues, which are responsible for the entire protein, carbohydrate and lipid metabolism in the liver.

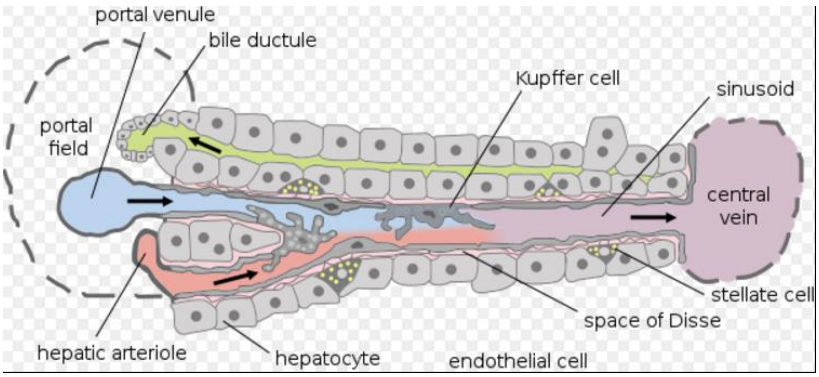

Figure1. Structure of hepatic sinusoid[5]

1.1.1.2 Cholesterol distribution through blood circulation

The transportation of cholesterol in the water-soluble blood circulation requires lipoproteins, because cholesterol and its stored form, cholesterol ester, are hydrophobic. Lipoproteins are macromolecular lipid-protein complexes with a hydrophobic core inside and a relatively hydrophilic coating outside (Fig2). The hydrophobic core contains triacylglycerol and

cholesterol esters, and the coating is a monolayer of phospholipids and cholesterol. Cholesterol and phospholipids make lipoproteins stable and soluble in water by aligning their hydrophilic heads outwards.

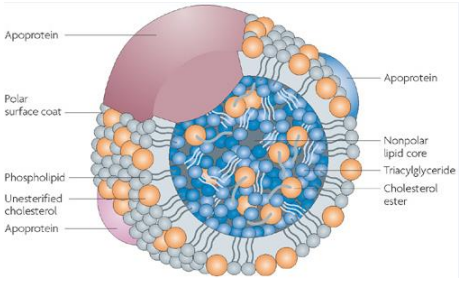

Figure2 Structure of lipoproteins[6]

Lipoproteins are labelled with different apolipoproteins. These apolipoproteins functions as fingerprints; therefore peripheral organs can recognize different lipoproteins and make use of the lipids inside. Based on density, size, apolipoprotein and composition, lipoproteins are classified into different groups or fractions: chylomicron, very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL)[7]. The main characteristics of these lipoprotein fractions are shown in Table 1.

Fraction	Density range (g/ml)	Diameter (nm)	Major lipids	Major apolipoproteins
Chylomicrons	<0.950	80-1000	Dietary TAG	B48, AI, AIV, C, E
VLDL	0.950-1.006	30-80	Endogenous TAG(from liver)	B100, C, E
LDL	1.019-1.063	20-25	Cholesterol and cholesteryl ester	B100
HDL	1.063-1.210	9-15	Cholesteryl ester and PL	AI, AII, C, E

Table1. Main characteristics of lipoprotein fractions[7]

VLDL has Apolipoprotein B100 (ApoB100) as the structural apolipoprotein and loads cholesterol, together with triacylglycerol (TG), with the facilitation of microsomal triglyceride transfer protein (MTTP). Through VLDL, cholesterol and TG is supplied from the liver to the peripheral tissues. Because the peripheral tissues extract the TG much faster than the cholesterol, VLDLs increase in density and transform into LDLs. LDLs then travel back to the liver and are recognized by LDL receptors. Pathologically, elevated levels of VLDL and LDL are the pre-requisite factor for developing atherosclerosis[8]. Atherosclerosis is a chronic inflammatory disease, which accounts for 50% of all deaths in westernized countries. The atherosclerotic process initiates from the accumulation of cholesterol-containing lipoproteins in the intima and decades later underlines the cause of most cardiovascular diseases, including myocardial infarction, stroke and ischemic gangrene[9].

HDLs, on the other hand, remove cholesterol and TG from the peripheral tissues and transport them back to the liver for excretion. HDL has Apolipoprotain A1 (ApoA1) as the

structural protein and loads cholesterol under the help of ATP-binding cassette transporter (ABCA1)[7].

1.1.1.3 Bile synthesis and enterohepatic circulation

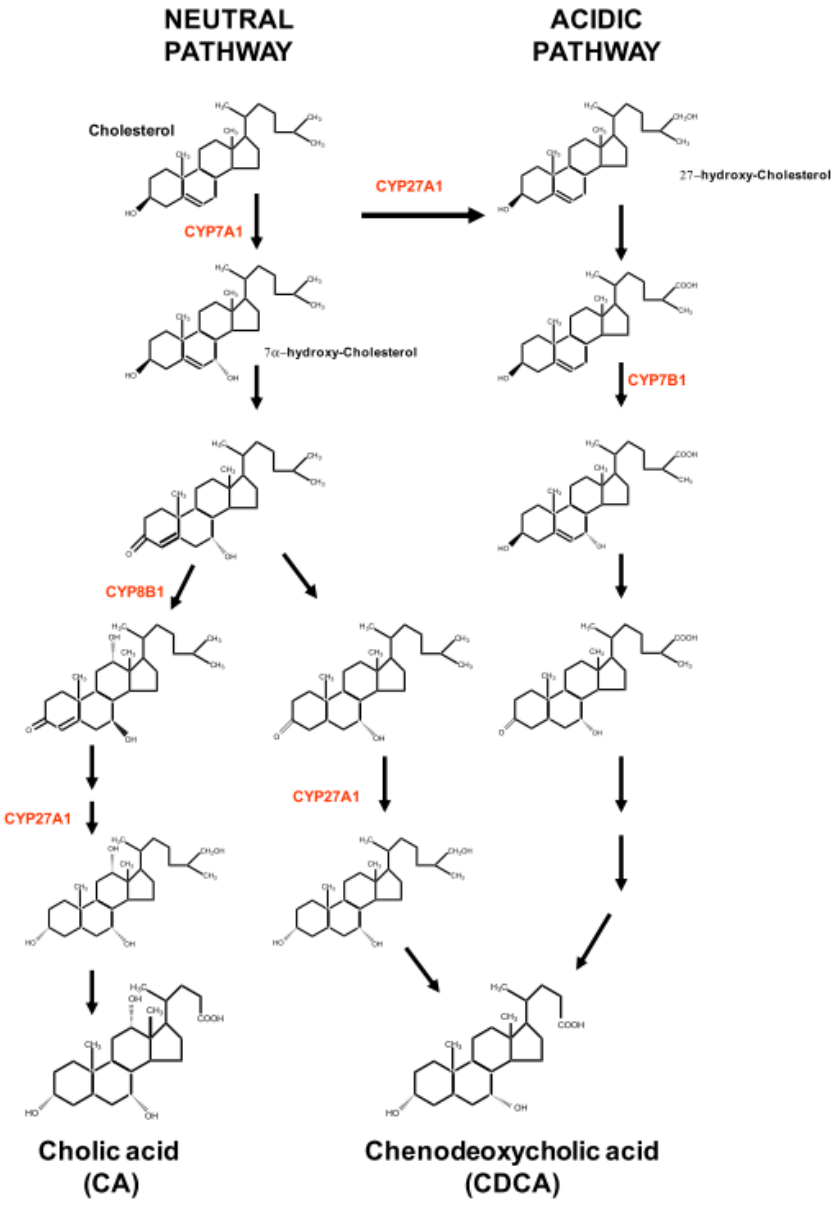

Figure 3 Bile acid syntheses[10]

The excessive cholesterol in the liver is converted into bile acids and later bile salts for excretion. Bile acids are synthesized in hepatocytes mainly by two pathways: neutral and alternative. As is shown in figure 3, cholesterol 7 alpha-hydroxylase (CYP7A1) is considered as the rate-limiting enzyme along the neutral pathway. This enzyme is regulated by a feed-back suppressive mechanism at the transcription level. When the end products of the neutral pathway, mainly cholic acid and chenodeoxycholic acid, are retentioned in the intestine, they stimulate the secretion of fibroblast growth factor 15/19 (FGF15/19). FGF15/19 is a hormone

which circulates back to the liver, signaling the hepatocytes by binding to its receptors and downregulating the gene expression of CYP7A. Several studies have shown that this down-regulation involves both c-Jun N-terminal kinase (JNKs) and extracellular signal-regulated kinases (ERK) signaling cascades[11]. The alternative pathway, also called the acidic pathway, is initiated by the enzyme sterol 27-hydroxylase (CYP27A1). Bile acid synthesis along the acidic pathway happens in mitochondria and the specific transporter Steroidogenic acute regulatory (StARD1) is required to help cholesterol pass the inner membrane. Studies have shown that this transportation is the rate-limiting step[10] along the acidic pathway and upregulation of StARD1 gene expression can markedly accelerate the bile synthesis through the acidic pathway[12].

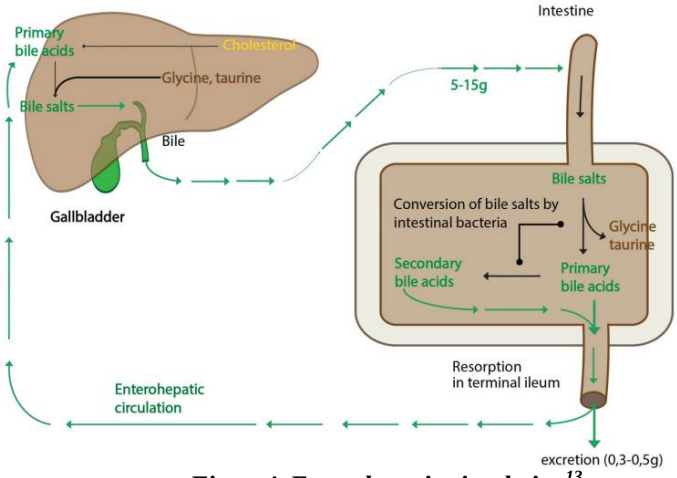

Figure4. Enterohepatic circulation[13]

Bile acids synthesized in the liver are called primary bile acids. They are then conjugated to either glycine or taurine to form bile salts. Bile salts are actively secreted out of the hepatocytes by ATP-binding cassette sub-family B member 11 (ABCB11), together with phospholipids by ABCB4, and cholesterol by ATP-binding cassette sub-family G member 5/8 (ABCG5/8)[14]. Bile salts function as an organic solvent and take phospholipids and cholesterol into the intestine via the bile ducts. In the intestine, part of the bile salts loose glycine and taurine and turn back into bile acids through bacteria digestion. These bile acids are defined as secondary bile acids and they are partially reabsorbed by the intestine and brought back to the liver via the portal vein. This circulation between liver and intestine is enterohepatic circulation (Fig 4). On the other hand, part of the cholesterol, secondary bile acids and bile salts are excreted into faces.

1.1.2 Cholesterol metabolism in the brain

Brain is a cholesterol-rich organ, which accounts for 20-25% of total cholesterol in the whole body[15]. Except for the contribution in cell membranes, cholesterol is also the raw material for myelin. Myelin forms an electrically insulating layer surrounding the axons of some nerve cells, which is fundamental for the efficient transmission of neuronal signals. Cholesterol metabolism in the brain is separated from the rest of the body by the blood-brain barrier (BBB). BBB is a highly selective permeability barrier composed by the brain-specific endothelial cells. This barrier prevents the cholesterol, among many other hydrophobic substances, from fluxing in or out of the brain. Although the brain, similar to all the other peripheral tissues, expresses the specific receptors for lipoproteins and ABCA1, there is no evidence showing that there is cholesterol exchange through lipoproteins between the brain and the blood circulation. Instead, several studies have shown by isotope tracking that the rate of cholesterol biosynthesis in the brain equals to the rate of cholesterol turnover in both developmental and adult periods, which indicates that all or at least the majority of cholesterol supply in the brain relies on *de novo* biosynthesis[16].

The excretory pathways of cholesterol from the brain share the similarity with the bile acid synthesis in the liver (Fig 3). It is initiated by CYP7A, however continued by 24-hydroxylase (CYP46A1) instead of CYP27A1. The corresponding product 24(S)-hydroxycholesterol diffuses through BBB and circulates to the liver for further excretion[16]. Defects of cholesterol metabolism in the brain have been implicated in several neurodegenerative diseases, such as Smith-Lemli-Opitz syndrome and Niemann-Pick type C disease[17].

1.1.3 Plant sterols

Plant sterols, as the name indicates, are found in the cell membranes of plants. The most common plant sterols in the human diet are campesterol and sitosterol[18]. Due to structure similarity (Fig 5), plant sterols are metabolized as cholesterol, but with far less efficiency in mammals. 2-5% of ingested plant sterols are absorbed at the small intestine, of which only 1 % reaches the blood circulation in the form of VLDL[19]. The consumption of plant sterols has increased during the past 10 years, mainly due to its potential benefit on cardiovascular health, because plant sterols has shown a strong effect on reducing plasma levels of cholesterol and LDL[20]. However, up-to-date, no

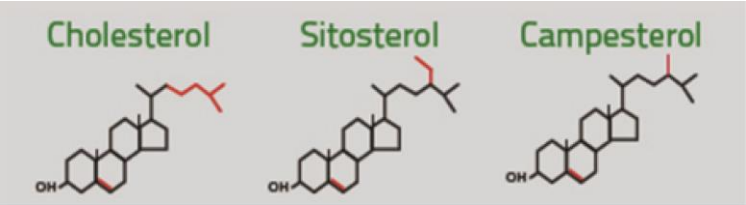

Figure5. Strcture of cholesterol, sitosterol and campesterol

association between plasma levels of plant sterols and risk of cardiovascular diseases has been detected[21]. At the same time, the side effect of plant sterols in the brains and its potential role in promoting neurological diseases has become an increasing concern. Several studies confirmed that plant sterols can pass BBB and accumulate in the brains in a concentration-dependent manner[22] and the molecular mechanism is suggested to involve the HDL receptors expressed on the endothelial cells of BBB[23]. From meta-analysis plant sterols have been linked to amyotrophic lateral sclerosis, but in vitro role of plant sterols in the setting of the central nervous system is still under discussions[23].

1.2 INSULIN PHYSIOLOGY

Glucose is the most direct energy source in the body. While many organs can also use fat and protein, the survival and normal function of the red blood cell and the brain is solely dependent on glucose supply. Insulin is a peptide hormone which plays an essential role in glucose clearance from the blood circulation by promoting its utilization primarily in the muscle, its short-term storage in the form of glycogen in the liver and its long-term storage in the form of fat in the adipose tissue.

1.2.1 Insulin secretion in pancreatic β cells

Insulin is secreted from β cells of the pancreatic islets in a glucose-dependent manner. Pancreatic islets, also known as islets of Langerhans, are the endocrine regions of pancreas. It consists of five major cell types: glucagon-producing α cells, insulin-producing β cells, somatostatin-producing δ cells, polypeptide-producing γ cells and ghrelin-producing Epsilon cells. After glucose stimulation, insulin secretion from the β cells follows a biphasic pattern. The first phase reaches its acute peak 4min after onset, and then drop back to a nadir around 8-10 min. The second phase is characterized by a gradually increasing rate of insulin release, which reaches the plateau 25-30min after glucose stimulation[24].

Three key steps are involved in the process of insulin secretion: insulin sensing, the triggering pathway and the amplification pathway (Fig 6). The glucose-sensing mechanism involves the glucose entry and glycolysis. Glucose transporter 2 (GLUT2) is highly expressed on pancreatic β cells and facilitate glucose transportation with high capacity but low affinity, which maintains the glucose level in and out of the β cells constantly equal. Thereafter, glucose goes through glycolysis, a series of enzymatic reactions to break down glucose and produce ATP. Disturbance of either GLUT2 or the rate-limiting enzyme of glycolysis, glucokinase, impairs the glucose sensing capacity of the β cells[25]. Subsequently the elevated ATP/ADP ratio triggers the ATP-sensitive potassium channel (K_{ATP} channel), which leads to depolarization of cell membrane and calcium influx through voltage-dependent calcium channels. Elevated concentration of calcium, thereafter, stimulates the exocytosis of insulin. This is the triggering pathway, which explains the first-phase of insulin secretion[26]. The amplification pathway, which corresponds to the second phase of insulin release, is to a great deal K_{ATP}-independent, but the mechanism is not fully understood yet. Insulin granules in β cells can be divided into different pools based on their readiness for secretion: reserved pool, docked pool, readily-releasable pool and immediately-releasable pool. Some researchers favor the hypothesis that the triggering pathway is largely responsible for the secretion of the

immediately-releasable pool and the amplification pathway is signaling to promote the readiness of insulin granules; in another word, to convert the reserve, docked and readily-releasable pools into its more ready status[27].

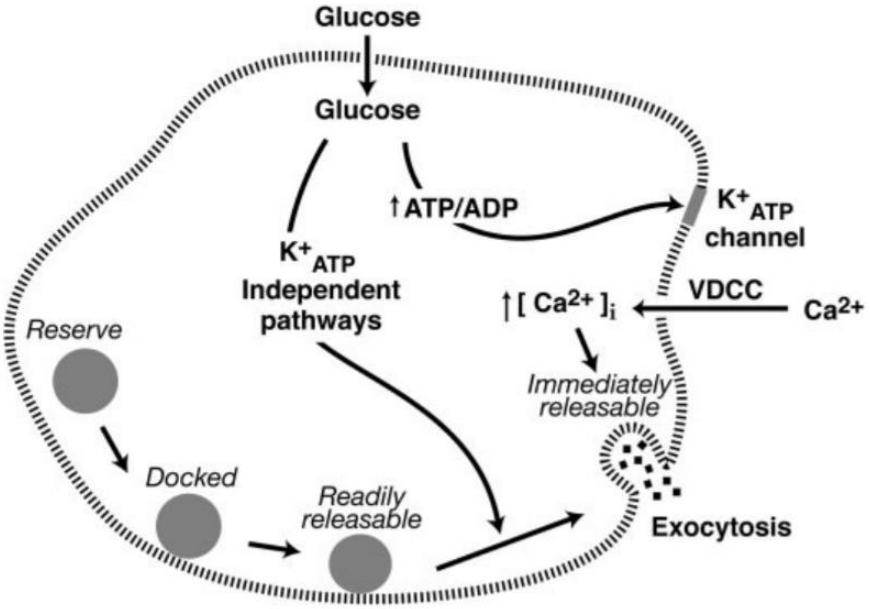

Figure6. Insulin granule pools and secretion pathway[27]

1.2.2 Insulin signaling for glucose utilization

As is shown in Figure 7, insulin signaling starts from binding to its specific receptor, insulin receptor, on the cell membrane. Following the insulin binding, the insulin receptor goes through auto-phosphorylation; therefore activates its intrinsic kinase and recruits insulin receptor substrate (IRS). IRS-1/2 plays a critical role in mediating two major signaling pathways. While the Ras/ERK pathway is promoting cell growth, the PI3K/Akt pathway, also known as the protein kinase B (PKB) pathway, is taking charge of a series of metabolic processes: 1) Akt promotes protein synthesis through regulating mTOR signaling pathway. 2) Akt enhances glucose uptake by accelerating the recycling of glucose transport type 4 (GLUT4). 3) In the muscle and the liver, Akt upregulates glycogenesis by mediating phosphorylation of glycogen synthase kinase (GSK3β). Glycogenesis is the biochemical process converting glucose into its short-term storage form, glycogen, after a series of enzymatic reactions during the well-fed state. In fasting state, glycogen in the liver breaks down into glucose and maintains the blood glucose level. During exercises, glycogen in the muscle supplies locally as the direct energy resource. 4) Fat is the long-term storage form of energy. In the liver and the adipose tissue, insulin signalling activates the transcription factor, Sterol regulatory element-binding protein 1 (SREBP-1), thus promotes fatty acid synthesis.

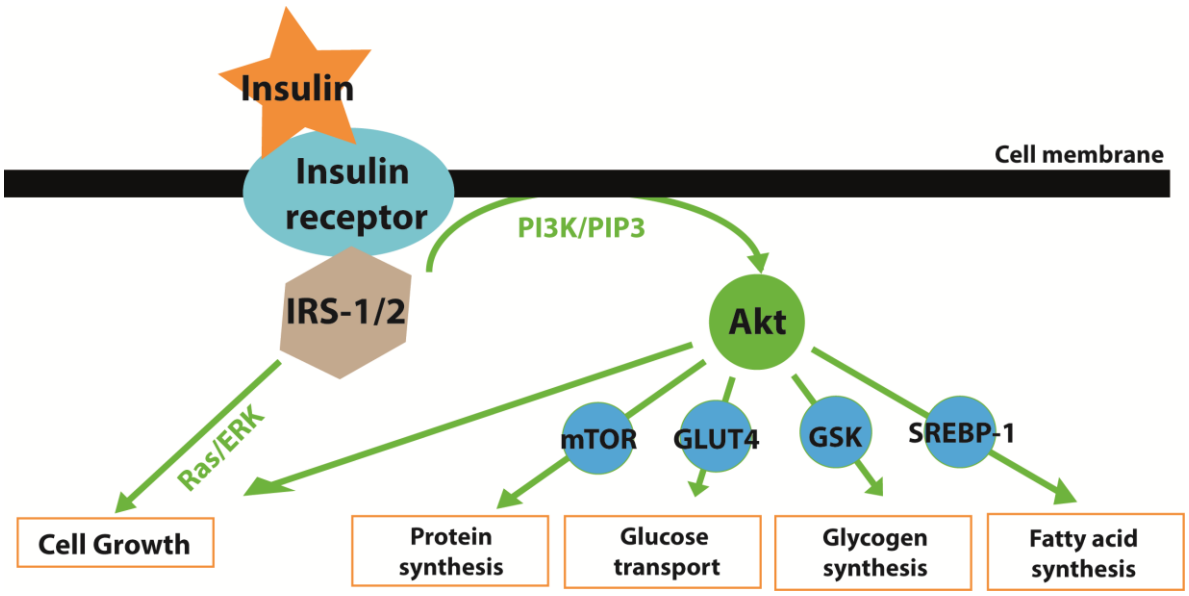

Figure7. Insulin signaling

1.2.3 Diabetes mellitus

Diabetes mellitus is a group of metabolic diseases characterized by elevated level of blood glucose over a prolonged period. Diabetes is a fast-growing public health issue and estimated to have a prevalence of 366 million people worldwide in 2030[28]. There are three major types of diabetes: Type I (insulin dependent) diabetes, type II (noninsulin dependent) diabetes and gestational diabetes. In this thesis, we focus on introducing type II diabetes, whose prevalence is driven by obesity and takes up 90% of all the diabetic population. The pathogenesis of type II diabetes is not fully understood yet. At the epidemiological level, there is a strong correlation between onset of type 2 diabetes and lipid accumulation outside the adipose tissues, for example in the liver and the muscle[29]. Furthermore, the impairment or loss of first-phase insulin secretion is observed and confirmed by many investigators[24]. At molecular level, several lipid species have been proven to impair both insulin secretion and sensitivity.

In pancreatic β cells, insulin secretion is dependent on elevated ATP/ADP ratio, which comes from glucose influx and glycolysis. In vitro studies on insulin-producing cell lines support the hypothesis that fatty acids affects insulin secretion by switching the balance between glycolysis and fatty acid oxidation inside the mitochondrial. For example, when islets are cultured in the glucose concentration which favors insulin secretion, the fatty acid oxidation is suppressed[30]. Reagents which inhibit the transportation of fatty acid into the mitochondrial for β oxidation boost insulin secretion from glucose-perfused islets[31]. Furthermore, capacity of insulin secretion is sensitive to cholesterol contents in the pancreatic β cells. The cholesterol homeostasis in the pancreatic β cells is finely balanced by *de novo* biosynthesis,

cholesterol efflux via HDL, and cholesterol uptake via LDL. Both up- and downregulation of the cholesterol content by manipulating these three pathways impair insulin secretion[32,33].

In the peripheral tissues, lipotoxicity interfere with insulin signaling at multiple levels. Ceramides, which is composed of sphingosines and a fatty acid, can inactivate Akt[34] and therefore block insulin actions in the metabolic perspective. Diacylglycerol (DAG), which consists of two fatty acid chains and a glycerol molecule, can alter the pattern of IRS phosphorylation and therefore decrease insulin signaling[35]. Furthermore, low level of inflammation has been detected in obese population, and proinflammatory cytokines such as tumor necrosis factor (TNF) contributes to insulin resistance by inactivating IRS[36].

1.3. PLATELET-DERIVED GROWTH FACTOR B (PDGFB)

PDGFB has been known as a mitogen for many cell types of mesenchymal or neuro-ectodermal origins. PDGFB is composed of a growth factor core domain which is necessary and sufficient for receptor binding and activation, and a retention motif at the C-terminal. The retention motif binds to heparin sulfate proteoglycan in the extracellular matrix, which defines the spatially distribution of PDGFB and affects its biological activity and action range. PDGFB forms homodimer (PDGF-BB), or heterodimer (PDGF-AB) by paring up with PDGFA. They initiate cell signalling by binding to platelet-derived growth factor receptors (PDGFRs). There are two forms of PDGFR, alpha and beta, and they also form homodimers *Pdgfrα/α*, *Pdgfrβ/β* and heterodimer *Pdgfrα/β*. *In vitro* studies have shown that PDGF-BB binds to *Pdgfrα/α*, *Pdgfrβ/β* and *Pdgfrα/β* while PDGF-AB binds to *Pdgfrα/α* and *Pdgfrα/β*. *In vivo* studies of PDGFB's interaction with its receptors are clear yet. So far PDGF-BB has only been confirmed to act on *Pdgfrβ/β*[37].

1.3.1 Pericytes and PDGFB retention motif knockout mouse model

PDGFB is mainly expressed by endothelial cells and it is well-known as a recruitment factor for pericytes in the field of vascular biology. As is shown in Figure 7, pericytes are contractile cells embedded in the basement membrane and outlining endothelial cells of capillaries and venules[38]. Proper coverage of pericytes along the blood vessels is very important for angiogenesis, vascular maturation and stability. Physiologically after being secreted from endothelial cells, PDGFB is highly concentrated at basement membranes because of its heparin sulfate proteoglycan binding domain (HSPG-BD). On the other hand, *Pdgfrβ* is expressed on pericytes. Through ligand-receptor attraction, pericytes are recruited to cover blood vessels.

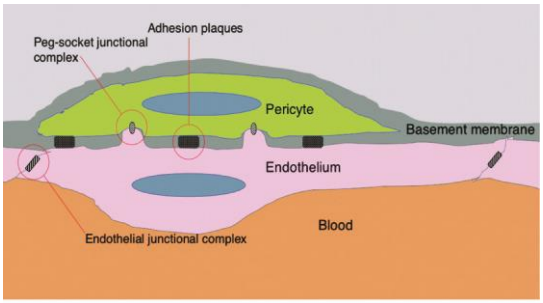

Figure7. Pericyte anatomy[36]

In order to study PDGFB signaling, a large number of mutations in *PDGFB* and *PDGFR* genes have been generated in mice. Unfortunately, knock-out mutants for *Pdgfb*, *Pdgfrα* or *Pdgfrβ* are lethal at embryonic state, which means that PDGFB signaling is crucial for embryonic development. In our study, we used the *Pdgfb*$^{ret/ret}$ mice model, in which the HSPG-BD is knocked-out while the growth factor core domain is not affected. PDGFB in *Pdgfb*$^{ret/ret}$ has slightly different signaling because of its local distribution. But since this gene

manipulation is much milder, these mice are viable. *Pdgfb*$^{ret/ret}$ has been intensively studied in BBB. BBB is a specific physical barrier mainly composed of endothelial cells to maintain the Central Nervous System (CNS) enclosed. As shown in Figure 8, when the retention motif is missing, PDGFB is no longer highly concentrated on the basement membrane, thus pericytes are partially detached and the BBB becomes leaky[39]. In liver however, this mice model has been poorly studied.

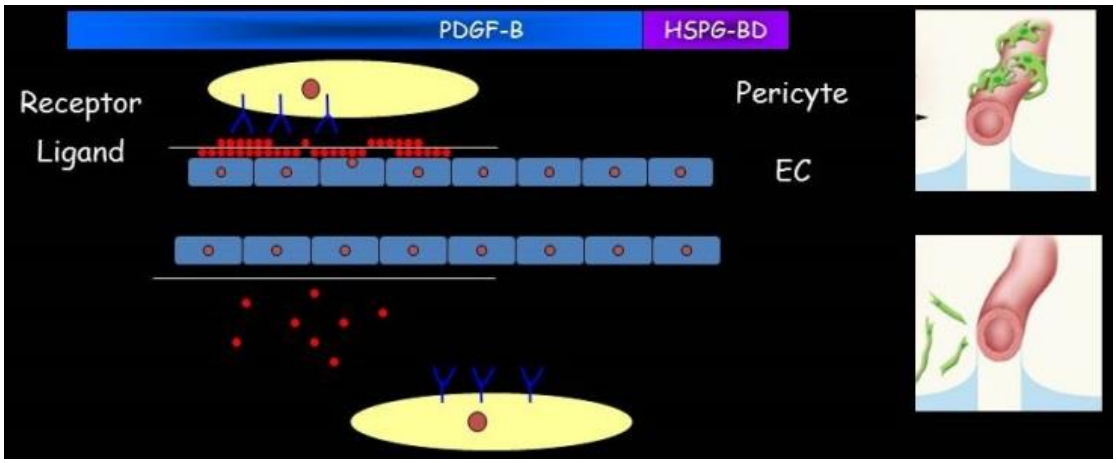

Figure 8. Mechanism of pericyte deficiency in Pdgfb$^{ret/ret}$

1.3.2 PDGFB signaling in the liver

Under physiological conditions, hepatic PDGFB is produced from portal veins[40]. From vascular perspective, PDGFB signalling in the liver is special, because PDGFB is not predominantly expressed from the sinusoidal endothelial cells, and the recruitment of liver pericytes is independent of PDGFB[41]. Hepatic PDGFB is well-known as a potent mitogen for HSCs and critically involved in fibrogenesis following injuries of different etiologies. In biliary diseases, PDGFB is detected in newly-proliferated cholangiocytes[40] and in non-biliary diseases, pathological PDGFB is suggested to be released from macrophages[42]. PDGFRα and PDGFRβ are expressed on HSCs. After binding to PDGFB, PDGFRs undergoes auto-phosphorylation and recruits its adaptor Grb2. Activation of PDGFRs transduces signals through a wide spectrum of pathways including COX2-PGs-cAMP, PI3K/PKB, Ras/ERK and ion channels. All these pathways serve to help HSCs survive or proliferate[43-45]. Proliferation of HSCs is the first step for HSC activation, which is followed by migration, trans-differentiation into a myofibroblastic cell type and production of extracellular matrix. *In vitro,* PDGFB promotes the proliferation, but not trans-differentiation, which indicates that the whole process of HSC activation also requires other stimuli such as reactive oxygen

species (ROS) and inflammatory cytokines[42]. However, overexpression of PDGFB in hepatocytes in vivo gives rise to liver fibrosis[44].

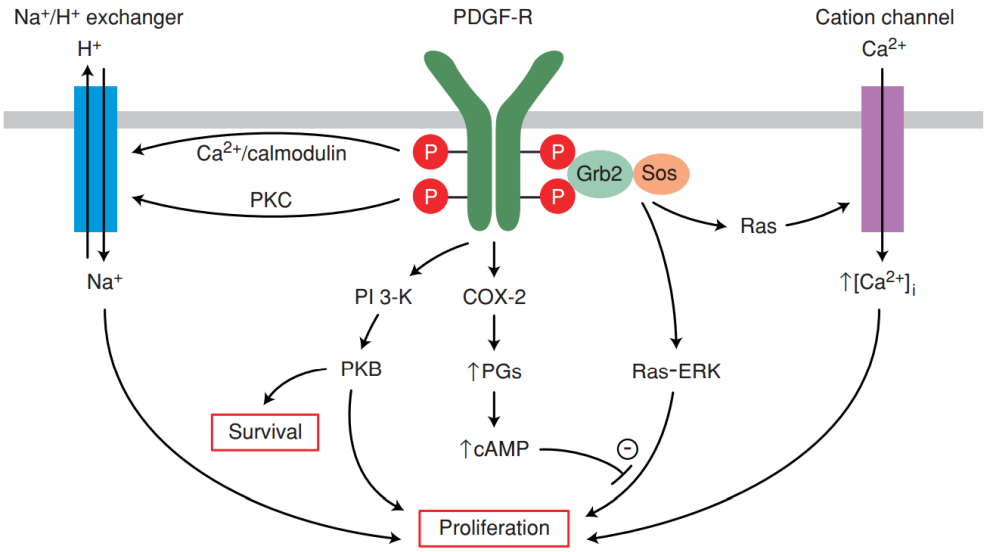

Figure 9. Mitogenic pathways of PDGF in hepatic stellate cells (HSCs)[43]

2. AIM OF THIS THESIS

PDGFB has been previously known as a major recruitment factor for pericytes, and therefore plays an important role in angiogenesis and normal vascular function. In our study, we used *Pdgfb*$^{ret/ret}$ to study the postnatal effect of PDGFB signaling in cholesterol and glucose metabolism.

In the brains, pericyte deficiency is confirmed in *Pdgfb*$^{ret/ret}$ and this leads to BBB leakage. BBB has been shown as an important barrier to keep the integrity of brain cholesterol metabolism. In **Paper I and II**, we used *Pdgfb*$^{ret/ret}$ as a BBB leakage model and observed how the cholesterol metabolism in the brain has changed.

In the liver, PDGFB is critically involved in fibrogenesis following liver injuries, but the physiological function of PDGFB is poorly investigated. In **Paper III**, we started from localizing PDGFB and PDGFRβ expression in the liver. Meanwhile, based on the observations in *Pdgfb*$^{ret/ret}$, we aimed to analyze the metabolic consequences of disrupted PDGFB signaling in the liver

Paper IV aimed to explore the role of PDGFB signaling in the pancreatic islets and try to decipher why *Pdgfb*$^{ret/ret}$ has faster glucose clearance and how PDGFB participates to regulate glucose metabolism.

3 PAPERS AND MAIN RESULTS

Since the material and method, the results and the discussions are presented in details in each paper/manuscript, only a short summary of the main findings are highlighted below.

3.1 PAPER I&II; ROLE OF BBB INTEGRITY IN BRAIN CHOLESTEROL METABOLISM

In the brain, PDGFB is the critical molecule for the recruitment of pericytes. In *Pdgfb*$^{ret/ret}$, pericyte coverage is reduced by 70% on the BBB, and this leads to increased permeability of a range of low- and high-molecular mass tracers. In our study, we used *Pdgfb*$^{ret/ret}$ as a model of defective BBB and studied the biological importance of BBB to cholesterol metabolism in the brain. As is summarized in Figure 10, when BBB is intact, cholesterol supply in the brain comes from de novo biosynthesis. Although endothelial cells of BBB express receptors for both HDL and ApoB-containing lipoproteins, cholesterol in the circulation cannot pass BBB through transcytosis (A). Pericytes have been shown to inhibit transcytosis across the BBB, and when pericytes are deficient in *Pdgfb*$^{ret/ret}$, cholesterol together with its analog plant sterols fluxes into the brain at a concentration-dependent manner (B). On the other side, replaced cholesterol from metabolic turnover is excreted out the brain in the form of 24-hydroxycholesterol (24-OHC). 24-OHC is an oxidized metabolite of cholesterol, and important for sending negative feedback to the *de novo* biosynthesis in the brain. Under physiological conditions, 24-OHC can pass BBB into the circulation through both passive and active transportation (A). In *Pdgfb*$^{ret/ret}$, a significantly stronger efflux of 24-OHC is speculated, as indicated by the reduced level of 24-OHC in the brain and less concentration difference in and out of the brain. Because the negative feedback from 24-OHC is reduced, the *de novo* biosynthesis of cholesterol in the brain is increased. On the contrary, plant sterols cannot be excreted by the same pathway, and therefore in *Pdgfb*$^{ret/ret}$, more plant sterols are accumulated in the brain.

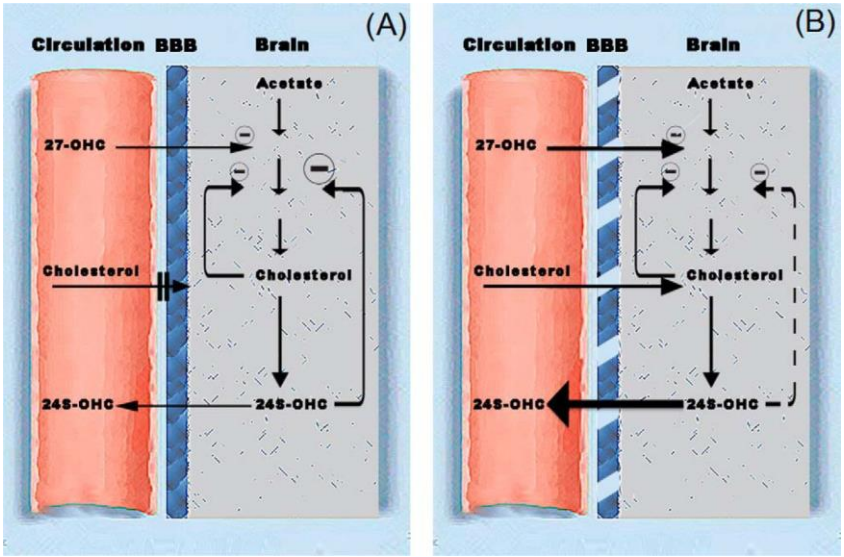

Figure 10. Theoretical models for metabolic consequences of brain cholesterol from defective BBB

3.2 PAPER III; ROLE OF PDGFB IN THE LIVER PATHOPHYSIOLOGICAL CHOLESTEROL REGULATION

PDGFB in the liver is an essential molecule for initiating fibrogenesis by activating HSCs following pathological stimuli. Under physiological senario, however, the role of hepatic PDGFB signaling is poorly investigated.

In Paper III, we started from visualizing the expression of PDGFB and its receptor PDGFRβ in the liver. In agreement with the previous study, we confirmed that PDGFB is expressed from the endothelial cells of portal veins. In addition, PDGFB is also expressed in the cholangiocytes of bile ducts in the portal area. This finding guided us to characterize in details the portal mesenchymal cells which express PDGFRβ. Using a panel of markers, we found that mesenchymal cells surrounding the portal endothelial cells are expressing ASMA but not PDGFRα, while mesenchymal cells surrounding the bile ducts are expressing PDGFRα but not ASMA. Both periductal and perivascular mesenchymal cells express desmin and colocalize with collagen deposit.

By using PDGFB knockouts and PDGFRβ overreactive mouse model, we detected a direct link between elevated PDGFB signaling and monocyte infiltration both generally in the liver and intensively clustered around the bile ducts. The clustering of monocytes around the bile ducts is defined as portal inflammatory infiltrate(PII) and is of great clinical interest due to its ubiqutous presence in biliary and non-biliary liver diseases. To further explore the postnatal effect of PDGFB, we used *Pdgfb*$^{ret/ret}$, where the hepatic PDGFB signaling is slightly elevated. In accordance to our expectation, general monocyte infiltration and PII also take the place in *Pdgfb*$^{ret/ret}$, and in parallel we found two other defects related to the bile ducts: bile duct dilation and deficiency of periductal mesenchymal cells.

Bile duct is the fundamental structure for bile excretion. *Pdgfb*$^{ret/ret}$ shows intolerance to high cholesterol diet and develops hepatic steatosis. However the lipid retention in the liver is not due to defects in bile duct function, but suppression of VLDL secretion. Later we proved that the VLDL suppression is mediated by PDGFB-induced inflammation represented by PII. When we high-jacked the inflammation by depleting neutrophils from the circulation, hepatic steatosis, together with PII and VLDL suppression, is normalized.

At last, this project finishes with a detailed profiling of the immune populations in the liver. We showed that CD8+ T cells, together with Interferon gamma, are significantly upregulated while NKT cells are decreased in *Pdgfb*$^{ret/ret}$.

3.3 PAPER IV; ROLE OF PLATELET-DERIVED GROWTH FACTOR B IN INSULIN SECRETION

It has been previously reported that *Pdgfb*$^{ret/ret}$ has faster glucose clearance due to increased insulin sensitivity in the liver[46]. At the beginning of Paper IV, we performed intraperitoneal glucose tolerance test on mice of different ages and at different time points during the day. Our results confirm that *Pdgfb*$^{ret/ret}$ clears glucose in a more efficient way, and this phenotype is not affected by age or the diurnal cycle. The previous study assumed that *Pdgfb*$^{ret/ret}$ has less efficiency of PDGFB signaling in the liver, in a similar way as confirmed on the BBB. It reported that the sinusoids in *Pdgfb*$^{ret/ret}$ are more permeable to fluorescent tracers, and the liver uptakes more glucose when challenged with high dose of insulin[46]. From Paper III, however, we showed that hepatic PDGFB signaling is slightly higher in the liver. We then performed intraperitoneal insulin tolerance test together with glucose uptake assay. Our results showed that *Pdgfb*$^{ret/ret}$ has similar level of insulin sensitivity in the peripheral tissues and the liver is not taking up more glucose compared with the control littermates. We explained the faster glucose clearance in *Pdgfb*$^{ret/ret}$ as a more efficient patter of insulin secretion. Although *Pdgfb*$^{ret/ret}$ showed less insulin contents in the pancreas and inclined to secrete less insulin during the whole process of intraperitoneal glucose tolerance test. However, the first phase of insulin secretion in *Pdgfb*$^{ret/ret}$ is significantly earlier and stronger compared to the control littermates.

To explore the mechanism how PDGFB signaling regulates insulin secretion, we first checked vasculature in pancreatic islets, which includes vessel density, pericyte coverage, perfusion and permeability. All of these parameters show no difference in *Pdgfb*$^{ret/ret}$, which indicates that PDGFB is not a critical molecule for pericyte maintenance in pancreatic islets and the difference in insulin secretion cannot be justified by vascular changes. Furthermore we examined the islet size, proliferation rate and ex vivo capacity of insulin secretion after glucose stimulation. All the results show no difference in *Pdgfb*$^{ret/ret}$, which suggests that it is a systematic effect, which leads to the earlier and stronger insulin secretion at its first phase. More investigations are needed to set up the link between the changes of PDGFB signaling in *Pdgfb*$^{ret/ret}$ and the insulin secretion pattern we observed.

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