Novel targets in the liver to treat cardiometabolic diseases

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CHAPTER 7

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Summary

Obesity is a growing worldwide epidemic, affecting more than 0.89 billion adults by 2022, and is projected to further increase by nearly 70% by 2035. Consequently, the incidence of obesity-induced cardiometabolic diseases has markedly increased, becoming a major health problem and severely impacting the quality of life and threatening long-term health outcomes worldwide, despite the availability of current therapeutic strategies. This highlights the urgent need for new treatment strategies. Therefore, in this thesis, we investigated novel therapeutic targets for combating cardiometabolic diseases, especially for metabolic dysfunction-associated steatotic liver disease (MASLD) and atherosclerotic cardiovascular disease (ASCVD). In **Chapter 1**, I have provided a general introduction on the link between obesity and cardiometabolic diseases, and on the pathology of MASLD and ASCVD. Also, the current available therapeutic strategies, including lifestyle and pharmacological interventions and their efficacy as well as limitations, were introduced. In addition, I described several promising novel therapeutic targets for MASLD and ASCVD. As liver is the central organ in regulating lipid metabolism and inflammation, strongly affecting the development of MASLD and ASCVD, these targets were mainly within the liver, including liver X receptor (LXR), hepatic lipase (HL), and ATP-binding cassette transporter A6 (ABCA6).

LXR activation is a promising therapeutic strategy for preventing/treating MASLD and ASCVD by enhancing cholesterol efflux from macrophages and preventing inflammation. However, synthetic LXR agonists also induce lipogenesis in hepatocytes, causing side effects, such as hepatic steatosis and hyperlipidemia. Desmosterol, which is converted by Δ24-dehydrocholesterol reductase (DHCR24) into cholesterol, is a potent endogenous LXR ligand in macrophages but not in hepatocytes. In **Chapter 2**, we investigated the effects of increasing desmosterol by DHCR24 inhibition on MASLD development. Here, by using APOE*3-Leiden.CETP mice, a well-established model for human-like lipoprotein metabolism that develops high-fat high-cholesterol diet-induced human-like MASLD characteristics, we reported that 8-week treatment with SH42, a specific and selective DHCR24 inhibitor, markedly increases desmosterol levels in liver and plasma, reduces hepatic lipid content and steatosis score, and decreases plasma fatty acid and cholesteryl ester concentrations. Flow cytometry showed that SH42 decreases liver inflammation by preventing Kupffer cell activation and monocyte infiltration. LXR deficiency completely abolished these beneficial effects of SH42. Together, inhibition of DHCR24 by SH42 prevents diet-induced hepatic steatosis and inflammation in a strictly LXR-dependent manner without causing hyperlipidemia. Finally, we also showed that SH42 treatment decreases liver collagen content and plasma alanine transaminase levels in APOE*3-Leiden.CETP mice. In conclusion, we anticipate that pharmacological DHCR24 inhibition may represent a novel therapeutic strategy for the treatment of MASLD.

Given the promising anti-inflammatory effect of DHCR24 inhibition via SH42 treatment on preventing MASLD, in **Chapter 3**, we next aimed to evaluate a potential protective effect of SH42 on atherosclerosis development in APOE*3-Leiden.CETP mice and LDL receptor knockout (*Ldlr*$^{-/-}$) mice, models for lipid- and inflammation-driven atherosclerosis following Western-type diet feeding, respectively. In both models, we again confirmed that SH42 increases desmosterol in liver and plasma without affecting plasma lipid levels. While reducing liver lipids in APOE*3-Leiden.CETP mice, and regulating populations of circulating monocytes in *Ldlr*$^{-/-}$ mice, 15- or 13-week treatment of SH42 did not attenuate atherosclerosis development in either model. This is possibly related to insufficient availability of systemically administered SH42 to macrophages within atherosclerotic plaques to exert anti-inflammatory/anti-atherosclerotic effects, which may be overcome by developing lesion macrophage-targeted DHCR24 inhibitory strategies in future studies.

In addition to targeting cholesterol metabolism and inflammation, remodeling of lipoproteins via accelerated hydrolysis of their triglycerides (TG) and/or phospholipids (PL) by lipases has emerged as a novel therapeutic strategy for ASCVD. Hepatic lipase (HL) is a liver-bound protein with both phospholipase and TG hydrolase activity and is involved in the catabolism of circulating lipoproteins. A gain-of-function HL variant, HL-E97G, has been identified in a French family, with selectively increased phospholipase activity, and individuals carrying HL-E97G have very low plasma lipid levels. Therefore, in **Chapter 4**, we aimed to evaluate the lipid-lowering and anti-atherogenic properties of HL-E97G versus wild-type HL (HL-WT) in APOE*3-Leiden.CETP mice, and to assess dependence of these effects on the LDLR pathway in *Ldlr*$^{-/-}$ mice. APOE*3.Leiden.CETP mice or *Ldlr*$^{-/-}$ mice received an intravenous injection of liver-targeted virus (i.e., AAV8) expressing either eGFP (control), HL-WT or HL-E97G while being fed pro-atherogenic diets. We found that HL-E97G largely decreases plasma total cholesterol exposure in APOE*3-Leiden.CETP mice, resulting at least in part from increased uptake of (V)LDL by the liver, accompanied by a marked decrease in atherosclerotic lesion size in the aortic root. Importantly, HL-E97G also strongly reduced plasma cholesterol exposure in *Ldlr*$^{-/-}$ mice, and decreased atherosclerotic lesion size in the aortic root and the aortic arch. In conclusion, HL-E97G strongly reduces plasma cholesterol levels by increasing the uptake of (V)LDL from plasma, to decrease atherosclerosis development in mice independently of the LDLR pathway. These data suggest that modulating HL function is a promising tool in patients with familial hypercholesterolemia.

Lipid transport between cells and organs is vital to maintain cellular/organ function and strongly influences the development of cardiometabolic diseases, with ATP-binding cassette (ABC) transporters playing key roles. The role ABCA1, ABCG1 and ABCG5/ABCG8 in regulating cholesterol transport/metabolism and combating cardiometabolic diseases has been well-studied. In addition to these extensively studied ABC transporters, emerging evidence suggests that additional ABC transporters are involved in the transport of cholesterol. Of these, ABCA6 has been considered as a novel target for regulating cholesterol

metabolism and related diseases. ABCA6 is mainly expressed by hepatocytes, and a missense variant of *ABCA6* rs77542162 has been previously identified to be associated with higher cholesterol in a Dutch population. In **Chapter 5**, we aimed to elucidate the underlying mechanisms of *ABCA6* missense variant-associated hypercholesterolemia and evaluate the consequences for atherosclerosis. To this end, we used the UK Biobank to not only confirm these findings but in addition reveal an association of rs77542162 with increased circulating non-HDL cholesterol and ApoB levels. We also revealed a directionally consistent non-significant increased risk of ischemic heart disease in a two-sample Mendelian randomization analysis using combined cohorts. Furthermore, we showed that hepatic ABCA6 gene expression negatively correlates with circulating very low-density lipoprotein (VLDL) remnant-cholesterol levels in humans, suggesting the liver is central in the effects on ABCA6 on cholesterol metabolism. Therefore, we next performed hepatocyte-specific *Abca6*-disruption by AAV-CRISPR technology in Western-type diet-fed APOE*3-Leiden.CETP mice. We found that *Abca6*-disruption increases cholesterol in hepatic plasma membranes and lowers LDLR abundance to decrease hepatic uptake of VLDL remnants, thereby increasing plasma non-HDL cholesterol and aggravating atherosclerosis development. *Abca6*-disruption did not affect non-HDL cholesterol in *Ldlr*$^{-/-}$ mice, confirming the cholesterol-increasing effect is mediated through LDLR reduction. In conclusion, ABCA6 facilitates hepatic LDLR-mediated lipoprotein clearance, and as such is a promising therapeutic target to treat hypercholesterolemia and protect against atherosclerosis.

Finally, in **Chapter 6**, results from the studies described in this thesis were summarized and discussed in the context of the current scientific literature. Overall, our findings revealed three novel therapeutic targets, including LXR, HL and ABCA6, for combating cardiometabolic diseases, especially for MASLD and ASCVD. Specifically, our findings indicated that desmosterol-induced LXR activation via DHCR24 inhibition suppresses inflammation to prevent MASLD, and enhances the phospholipase activity of HL and the abundance/activity of hepatic ABCA6 may reduce atherogenic lipids to decrease ASCVD. Collectively, this thesis provides valuable insights into the therapeutic potential of these novel targets, facilitating further investigation into their underlying functional mechanisms and hopefully accelerating their translational applications in humans for combating cardiometabolic diseases.