Intervention in hepatic lipid metabolism : implications for atherosclerosis progression and regression

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Chapter 8

General discussion and perspectives

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GENERAL DISCUSSION AND PERSPECTIVES

Atherosclerosis is the underlying cause of most cardiovascular diseases [1]. Hypercholesterolemia holds a key role in the development of atherosclerosis and is a causative factor for coronary artery disease[2]. Lowering of VLDL- and LDL-cholesterol levels leads to a reduction in cardiovascular morbidity and mortality[3]. In contrast, high levels of HDL-cholesterol are associated with a decreased risk of cardiovascular disease[4]. This dissertation is dedicated to the regulation of lipid metabolism pathways and its subsequent effects on atherosclerotic lesion progression and regression.

1. Hepatic lipid metabolism and pharmaceutical interventions in the liver

Atherosclerosis is a liver disease of the heart[5]. Liver is considered as the major organ with significant therapeutic importance for the maintenance of metabolic homeostasis[6]. The first part of the thesis focuses on the hepatic lipid metabolism and the pharmaceutical interventions in the liver.

1.1 Nuclear receptors in the liver: potential targets for pharmaceutical interventions

Nuclear receptors (NRs) are ligand-activated transcription factors that act as sensors for a broad range of natural and synthetic ligands. A growing number of NRs have been identified as regulators in lipid metabolism and energy utilization[7]. Moreover, NRs control hepatic inflammation, regeneration, fibrosis, and tumor formation. Therefore, NRs are crucial for understanding the pathogenesis and pathophysiology of a wide range of hepatic disorders. Targeting NRs and their regulations in the liver offers new perspectives for the treatment of metabolic diseases[8].

In **Chapter 2**, we have composed the hepatic cell type-specific expression profile of NRs to provide the most complete quantitative assessment of NRs distribution in the liver reported to date. We have identified several liver-enriched orphan NRs as potential novel targets for pharmaceutical interventions. Specifically, members of the orphan receptor family COUP-TFs showed distinguished distributions in liver. COUP-TF3 was abundantly and exclusively expressed in liver parenchymal cells, while the other two members, COUP-TF1 and COUP-TF2, were expressed exclusively in non-parenchymal cells. Despite the moderate to high expression level in the liver, the physiological functions of COUP-TFs have not been fully exploited, and the ligand for COUP-TFs has not been identified. Our study suggests a physiologic important role of COUP-TF3 in hepatocytes, and that of COUP-TF2 in endothelial cells and macrophages. Previous studies have shown that the activity of COUP-TFs is associated with the transcriptional regulation of a number of genes expressed mainly in the liver[9]. COUP-TF2 and COUP-TF3 have been generally considered to be repressors or regulators for transcription of NRs such as RARs, TRs, PPARs, and HNF4alpha[10]. The cellular distribution pattern from the present study suggests that COUP-TF3, given its high expression in liver parenchymal cells, may have a potential role in hepatic lipid and xenobiotic metabolism regulation via cross-talking with other liver-enriched NRs. Our study identifies COUP-TF2 as highly expressed in endothelial cells, which is in

accordance with published data upon the role of COUP-TF2 in angiogenesis and generation of haematopoietic cell clusters[11]. In addition, COUP-TF2 was shown to have a potential role in regulation of cholesterol homeostasis[12]. The observation from our current study that the expression of COUP-TF2 is as high as NGFIB in Kupffer cells raises interest to further investigate whether COUP-TF2 is involved, similarly as NGFIB, in lipid metabolism in macrophages.

We used real-time quantitative PCR to gain the expression profiles of NRs. It is a standardized method in the NR field to characterize the expression pattern of individual receptors in tissue or cells. It provides a simple but powerful way to obtain comprehensive understanding of the distribution and biological functions of NRs[13]. Real-time quantitative PCR has been applied in numerous studies to profile the expression pattern of NRs in tissues representing diverse anatomical systems under various pharmacological conditions and genotypes[14,15]. However, because NRs are transcription factors, the regulation of NRs are not only at the mRNA level, but also on the protein activity level. Therefore, it is important to further extend the gene expression profiles of NRs into their protein expression and/or functionality profiles by using immunohistochemical staining or flow cytometry. Furthermore, in addition to the hepatic endothelial cells and Kupffer cells, which were investigated in the current study, hepatic stellate cells are also major repositories for lipids, being activated during chronic liver injury and mediating the fibrotic response[16,17]. It is thus interesting to include in future studies the hepatic stellate cells when investigating gene expression profiles of NRs in the liver.

1.2 Niacin regulates hepatic lipid metabolism and CETP expression

The use of statins in patients with high risk for cardiovascular disease (CVD) has resulted in a 30-40% decrease in clinical events in the last couple of decades. However, despite of a marked reduction (up to 60%) in LDL-C, about 30% of patients continue to have CVD events[18]. Therefore, HDL has become the next promising therapeutic target. The anti-dyslipidemic drug niacin, also known as nicotinic acid, not only lowers plasma levels of pro-atherogenic lipids/lipoproteins[19], but also is the most effective agent available to increase HDL-cholesterol[20,21]. Several clinical trials have shown that niacin reduces cardiovascular disease and myocardial infarction incidence, providing an emerging rationale for the use of niacin in the treatment of atherosclerosis[22,23]. Only recently, it has been shown that niacin also reduces the hepatic expression and plasma levels of the pro-atherogenic CETP[24]. In **Chapter 4** we investigated the mechanism underlying the CETP-lowering effect of niacin. We propose that the primarily reduced hepatic cholesterol accumulation via the lipid-lowering effect of niacin leads to attenuated hepatic inflammation, and thus less macrophage infiltration into and/or increased macrophage emigration out of the liver. The decreased amount of hepatic macrophages, which are significant contributors of CETP, leads to an overall reduction in hepatic CETP expression and a lower plasma CETP level. In conclusion, we have shown that niacin does not directly alter macrophage CETP expression, but attenuates the liver inflammation and macrophage content in response to its primary lipid-lowering effect, which leads to a decrease in hepatic CETP expression and the plasma CETP mass. Our study sheds a new light on the mechanism underlying the CETP-lowering effect of niacin in CETP transgenic mice.

Niacin receptor GPR109A is highly expressed in adipose tissue. Interestingly, adipose tissue appears to be a highly conserved site of CETP expression across

species. Adipose tissue CETP makes a major contribution to CETP in the circulation, reduces HDL, and increases non-HDL cholesterol levels. Adipose tissue CETP plays a local role in adipocyte cholesteryl ester accumulation from HDL, indicating a novel role of CETP in modulating adiposity. It is thus of interest to further investigate the potential regulation effects of niacin on adipose tissue CETP expression.

Hernandez *et al*[25] demonstrated for the first time the critical role of CETP in niacin-mediated HDL-elevation by using CETP tg mice and suggested that transgenic mice expressing the human CETP could be a useful animal model for studying the established HDL-regulating effect of niacin. However, in the current study we did not observe the HDL-raising effect of niacin in CETP Tg mice. This might be due to diet differences. Hernandez *et al* used regular chow diet supplemented with niacin, while we used WTD diet which contains more fat and 0.25% cholesterol that has been shown to upregulate CETP expression. Furthermore, although niacin significantly increased hepatic apoA-I expression in our study, the hepatic expression of ABCA1, which is involved in apoA-I-mediated cholesterol efflux, significantly decreased by 45% at the same time upon niacin treatment, which can ultimately result in unchanged HDL formation in plasma. Interestingly, our observations corroborate recent data from Li *et al*[26]*,* who showed that niacin-mediated activation of GPR109A in liver led to reduced hepatocyte ABCA1 expression and thus an unexpectedly reduced plasma HDL-C level in C57BL/6J mice. The current study was set out to investigate the mechanism underlying the hepatic and plasma CETP-lowering effect of niacin in mice, instead of determining the mechanism of HDL-C elevation by niacin. This mechanistic approach in a mouse model thus differs from a clinical investigation.

Recent unexpected termination of AIM-HIGH Clinical Trial on niacin and HDL hypothesis has drawn much attention. AIM-HIGH is the first large randomized trial to evaluate the effect of niacin on cardiovascular events among statin-treated patients with established atherosclerotic cardiovascular disease who are at the desired level for LDL-C but who have residual abnormalities in HDL-C and triglycerides. It was meant to test whether adding high dose, extended-release niacin to a statin (simvastatin) is better than a statin alone in reducing long-term cardiovascular events in participants[27]. The trial was started in September 2005, but was stopped early due to the lack of incremental benefit on cardiovascular risk reduction in the extended-release niacin plus simvastatin treatment group over simvastatin alone. At this time, the FDA has made no new conclusions or recommendations regarding the use of extended-release niacin alone or in combination with simvastatin or other statins[28]. The lack of additional effect of niacin on cardiovascular events is unexpected and a striking contrast to the results of previous trials and observational studies, despite that niacin in this trial produced the predicted effects on all lipid parameters measured including increasing HDL levels by 20% and reducing triglycerides by around 25%[29]. The early termination of this trial raised questions about the suggested beneficial effects of the HDL-elevation hypothesis[30]. However, the AIM-HIGH study population was not a very high-risk population because the patients were already on statin-treatment and their LDL-cholesterol level was already well controlled. The study population in this trial does not represent all patient populations in whom the importance of treating low HDL and lowering triglycerides with Niaspan may be significant. Therefore, the relevance of these results to patients outside the AIM-HIGH study population is

currently unknown and it would be premature to extrapolate these results with Niaspan to a broader patient population at this time[31].

The clinical use of niacin has been limited due to the cutaneous flushing effect, which is mediated by the niacin receptor GPR109A in the skin. Therefore, in **Chapter 5**, we assessed the properties of two partial agonists for GPR109A and compared them to niacin. We show that these two GPR109A partial agonists of the pyrazole class are promising drug candidates to achieve the beneficial lipid-lowering effects while they successfully avoid the unwanted flushing side effect. Interestingly, our flushing results are somewhat complicated due to the unexpected skin temperature increase observed in the C57BL/6 mice treated with the vehicle DMSO. DMSO is one of the most common solvents used for *in vivo* administration of water-insoluble substances. In literatures, a wide range of pharmacological effects of DMSO has been documented in both animal and human experimental models, including membrane penetration and vasodilation[32,33,34]. It is proposed that DMSO provokes a histamine-like response, possesses potent histamine-liberating properties, and increases blood flow[35]. Despite being frequently used as a solvent in biological studies and as a vehicle for drug therapy, the undesirable side-effects of DMSO both *in vivo* and *in vitro* should be taken into consideration[36].

2. Atherosclerotic lesion regression and mouse models

The second part of the thesis focuses on the concept of atherosclerotic lesion regression, shedding insights in the role of LXR activation and application of mouse models in regression studies.

While numerous studies have been dedicated to inhibit the progression of atherosclerosis, recent attention has been focused on reversing atherosclerosis, which is the regression of existing atherosclerotic plaque. In previous studies, a dramatic regression of large advanced lesion was achieved with or without LXR agonist[37,38] by utilizing surgical aorta transplantation into wildtype mice. This procedure leads to a rapidly improved milieu for the atherosclerotic lesions. In another mouse model, apoE*3Leiden mice, LXR agonist were also shown to promote regression of moderate lesions[39]. In both **Chapter 6** and **Chapter 7** we investigated other different mouse models to explore technically easier and more efficient approaches for plaque regression.

In **Chapter 6** we first fed LDLr$^{-/-}$ mice with high-fat high-cholesterol Western-type diet (WTD) for 6 weeks to develop atherosclerotic plaques. Subsequently, a group of mice was sacrificed to obtain baseline data, whilst the rest of the mice were switched to a low-fat cholesterol-free chow diet without or with LXR agonist T0901317 supplementation for 3 weeks. Unexpectedly, T-0901317 supplemented in regular chow diet largely elevated plasma cholesterol level, and thus the atherosclerotic plaque continued its progression. Therefore, we set out to evaluate the effects of LXR agonist on plaque regression in C57BL/6 mice. The C57BL/6 mouse model has been used to study diet-induced atherosclerosis[40,41,42]. This murine model of atherogenesis represents an alternative to the use of genetically modified mice with impaired lipoprotein clearance, i.e. LDLr$^{-/-}$ mice, for the evaluation of anti-hyperlipidemic agents, including LXR agonists[43]. Here we fed C57BL/6 mice with cholate-containing cholesterol-enriched atherogenic diet for 16 weeks to induce atherosclerotic plaque development. Subsequently, a group of mice were sacrificed to obtain baseline data, whilst the remainder of the mice was

switched to low-fat cholesterol-free chow diet without or with LXR agonist T0901317 supplementation for 3 weeks. In contrast to data from $LDL^{-/-}$ mice, in C57BL/6 mice LXR agonist supplemented in regular chow diet successfully reduced the diet-induced hypercholesterolemia rapidly. LXR agonist not only reduced plasma (V)LDL cholesterol levels, but also significantly increased HDL level, leading to a 10-fold increased ratio between antiatherogenic HDL and proatherogenic (V)LDL level. This indicates that LXR agonist, together with chow diet, rapidly improved the plasma lipoprotein profile and induced a regression environment in C57BL/6 mice.

LXR agonist exhibited opposite effects on plasma lipoprotein and lesion progression in $LDLr^{-/-}$ and C57BL/6 mice, and we propose that this is due to the absence of LDL receptor and thus impaired LDL clearance in $LDLr^{-/-}$ mice. The LDL receptor-mediated cholesterol uptake and feedback regulation are crucial when evaluating LDL-lowering effects of compounds[44]. Studies have show that when LDL receptor gene was transferred back into hypercholesterolemic $LDLr^{-/-}$ mice to restore sustained expression of the LDLr protein in the liver, there was drastic reduction of plasma cholesterol and non-HDL cholesterol levels persistently and pronounced regression of advanced atherosclerotic[45,46]. Thus, it is proposed that C57BL/6 mice is a better model than $LDLr^{-/-}$ to evaluate (V)LDL-lowering induced atherosclerotic regression.

However, the limitation of the C57BL/6 mouse model is that the atherosclerotic plaque induced by an atherogenic diet was relatively small (~50 x 10^3 μm^2 in the aortic root), and there was hardly any lesion developed in the aortic arch or the descending aorta yet. Thus, the effect of LXR-agonist in this study was limited to diminishing early atheromatous lesion with small size and less complexity. It is thus important to further investigate the potential of LXR activation on diminishing larger advanced atherosclerotic plaque in both the aortic root and descending aorta.

In **Chapter 7** we used bone marrow transplantation technique, reconstituting $ApoE^{-/-}$ mice with bone marrow from wildtype mice, to restore apoE function in macrophages and normalize plasma lipoprotein profiles. This is a novel mouse model with chow diet feeding, providing an alternative model to investigate atherosclerotic plaque regression without robust surgical measures. We performed this regression study in parallel on initial and advanced lesions. We show that the bone marrow transplantation technique leads to successful plaque regression of both initial and advanced lesions. The current mouse model is thus a good model to study atherosclerosis regression in different stages of the disease. However, in initial lesions, a 45% reduction in lesion size was observed with chow diet alone, and even a 71% reduction with LXR agonist treatment, whilst in advanced lesions there was a more limited 23% reduction with chow diet alone, and a 36% reduction with LXR activation. This can be explained by the difference in plaque composition and characteristics between initial and advanced lesion. Initial lesions contain primarily cholesterol-filled macrophages covered by a thin cap, while in advanced lesion, SMCs and extracellular matrix products comprise the major structural components of the atherosclerotic plaques, covered by a thick fibrous cap[47], which make the advanced lesions more difficult to be modulated during the regression process.

Interestingly, our lesion composition analysis showed that the reduction of total lesion size during regression was primarily due to a reduction of the macrophage content in the plaque. However, the fate of the macrophages during lesion

regression is currently still under debate. It has been proposed that regression is not merely a rewinding of progression, but instead involves induction of CCR7 expression, a mediator of leukocyte emigration, leading to the emigration of the lipid-loaded macrophages, followed by the initiation of influx of healthy phagocytes that mobilize necrotic debris and all other components of advanced plaques[48]. Interestingly, Ye *et al* showed that macrophage infiltration into pre-existing advanced lesions was limited, likely because of the formation of fibrous caps[49]. In contrast, Potteaux *et al* also showed that regression of atherosclerosis after apoE complementation in ApoE$^{-/-}$ mice did not involve migratory egress of macrophages from plaques or induction of CCR7. Instead, marked suppression of monocyte recruitment coupled with a stable rate of apoptosis accounted for loss of plaque macrophages, suggesting that therapies to inhibit monocyte recruitment to plaques may constitute a viable strategy to reduce plaque macrophage burden than attempts to promote migratory egress[50]. Further research is necessary to establish the processes and mechanisms underlying the diminished macrophage content during regression in our experimental mouse model.

In conclusion, the second part of this thesis, focusing on novel mouse models to induce atherosclerotic lesion regression, shows that 1) intact LDL receptor function is crucial to overcome LXR-induced hyperlipidemia as to achieve plaque regression; 2) ApoE$^{-/-}$ mice reconstituted with bone marrow from C57BL/6 mice represents a promising mouse model with chow diet feeding to study the regression of atherosclerosis, providing an alternative model to investigate plaque regression without robust surgical measures; and 3) rapidly optimized plasma lipoprotein profiles, combined with LXR agonist treatment, induced favorable gene expression profiles, leading to regression of both initial and more advanced atherosclerotic plaques.

REFERENCES

1. Boehm M, Nabel EG. The cell cycle and cardiovascular diseases. Prog Cell Cycle Res. 2003;5:19-30.
2. Toutouzas K, Drakopoulou M, Skoumas I, Stefanadis C. Advancing therapy for hypercholesterolemia. Expert Opin Pharmacother. 2010;11:1659-1672.
3. Paras C, Hussain MM, Rosenson RS. Emerging drugs for hyperlipidemia. Expert Opin Emerg Drugs. 2010;15:433-451.
4. Lee JM, Choudhury RP. Atherosclerosis regression and high-density lipoproteins. Expert Rev Cardiovasc Ther. 2010;8:1325-1334.
5. Davis RA, Hui TY. George Lyman Duff Memorial Lecture: atherosclerosis is a liver disease of the heart. Arterioscler Thromb Vasc Biol. 2001;21:887-898.
6. Marchesini G, Moscatiello S, Di Domizio S, Forlani G. Obesity-associated liver disease. J Clin Endocrinol Metab. 2008;93:S74-S80.
7. Schulman IG. Nuclear receptors as drug targets for metabolic disease. Adv Drug Deliv Rev. 2010;62:1307-1315.
8. Trauner M, Halilbasic E. Nuclear receptors as new perspective for the management of liver diseases. Gastroenterology. 2011;140:1120-1125.
9. Lazennec G, Kern L, Valotaire Y, Salbert G. The nuclear orphan receptors COUP-TF and ARP-1 positively regulate the trout estrogen receptor gene through enhancing autoregulation. Mol Cell Biol. 1997;17:5053-5066.
10. Park JI, Tsai SY, Tsai MJ. Molecular mechanism of chicken ovalbumin upstream promoter-transcription factor (COUP-TF) actions. Keio J Med. 2003;52:174-181.
11. You LR, Lin FJ, Lee CT, DeMayo FJ, Tsai MJ, Tsai SY. Suppression of Notch signaling. by the COUP-TFII transcription factor regulates vein identity. Nature. 2005;435:98-104.
12. Myers SA, Wang SC, Muscat EO. The chicken ovalbumin upstream promoter-transcription factors modulate genes and pathways involved in skeletal muscle cell metabolism. J Biol Chem. 2006;281:24149-24160.
13. Bookout AL, Mangelsdorf DJ. Quantitative real-time PCR protocol for analysis of nuclear receptor signaling pathways. Nucl Recept Signal. 2003;1:12.
14. Bookout AL, Jeong Y, Downes M, Yu RT, Evans RM, Mangelsdorf DJ. Anatomical profiling of nuclear receptor expression reveals a hierarchical transcriptional network. Cell. 2006;126:789-799.
15. Yang X, Downes M, Yu RT, Bookout AL, He W, Straume M, Mangelsdorf DJ, Evans RM. Nuclear receptor expression links the circadian clock to metabolism. Cell. 2006;126:801-810.
16. Mallat A, Lotersztajn S. The liver x receptor in hepatic stellate cells: a novel antifibrogenic target ? J Hepatol. 2011; Epub ahead of print.
17. Anty R, Lemoine M. Liver fibrogenesis and metabolic factors. Clin Res Hepatol Gastroenterol. 2011;35 Suppl 1:S10-20.
18. Asztalos BF. High-density lipoprotein particles, coronary heart disease, and niacin. J Clin Lipidol. 2010;4:405-410.
19. Carlson LA. Niaspan, the prolonged release preparation of nicotinic acid (niacin), the broad-spectrum lipid drug. Int J Clin Pract. 2004;58:706-713.
20. Brooks EL, Kuvin JT, Karas RH. Niacin's role in the statin era. Expert Opin Pharmacother. 2010;11:2291-2300.
21. Al-Mohaissen MA, Pun SC, Frohlich JJ. Niacin: from mechanisms of action to therapeutic uses. Mini Rev Med Chem. 2010;10:204-217.
22. Lee JM, Robson MD, Yu LM, Shirodaria CC, Cunnington C, Kylintireas I, Digby JE, Bannister T, Handa A, Wiesmann F, Durrington PN, Channon KM, Neubauer S, Choudhury RP. Effects of high-dose modified-release nicotinic acid on atherosclerosis and vascular function: a randomized, placebo-controlled, magnetic resonance imaging study. J Am Coll Cardiol. 2009;54:1787-1794.
23. Taylor AJ, Villines TC, Stanek EJ, Devine PJ, Griffen L, Miller M, Weissman NJ, Turco M. Extended-release niacin or ezetimibe and carotid intima-media thickness. N Engl J Med. 2009;361:2113-2122.
24. Van Der Hoorn JW, De Haan W, Berbée JF, Havekes LM, Jukema JW, Rensen PC, Princen HM. Niacin increases HDL by reducing hepatic expression and plasma levels of cholesteryl ester transfer protein in APOE*3Leiden.CETP mice. Arterioscler Thromb Vasc Biol. 2008;28:2016-2022.
25. Hernandez M, Wright SD, Cai TQ. Critical role of cholesterol ester transfer protein in nicotinic acid-mediated HDL elevation in mice. Biochem Biophys Res Commun. 2007;355:1075-1080.
26. Li X, Millar JS, Brownell N, Briand F, Rader DJ. Modulation of HDL metabolism by the niacin receptor GPR109A in mouse hepatocytes. Biochem Pharmacol. 2010;80:1450-1457.

27. AIM-HIGH Investigators. The role of niacin in raising high-density lipoprotein cholesterol to reduce cardiovascular events in patients with atherosclerotic cardiovascular disease and optimally treated low-density lipoprotein cholesterol: baseline characteristics of study participants. The Atherothrombosis Intervention in Metabolic syndrome with low HDL/high triglycerides: impact on Global Health outcomes (AIM-HIGH) trial. Am Heart J. 2011;161:538-43.
28. Food and Drug Administration. FDA statement on the AIM-HIGH trial. May 26, 2011.
29. National Institutes of Health. NIH stops clinical trial on combination cholesterol treatment [press release]. May 26, 2011.
30. AIM-HIGH Investigators. The role of niacin in raising high-density lipoprotein cholesterol to reduce cardiovascular events in patients with atherosclerotic cardiovascular disease and optimally treated low-density lipoprotein cholesterol Rationale and study design. The Atherothrombosis Intervention in Metabolic syndrome with low HDL/high triglycerides: Impact on Global Health outcomes (AIM-HIGH). Am Heart J. 2011;161:471-477.
31. Wise J. Trial of niacin alongside statin is stopped early. BMJ. 2011; 342:d3400 doi: 10.1136/bmj.d3400
32. Colucci M, Maione F, Bonito MC, Piscopo A, Di Giannuario A, Pieretti S. New insights of dimethyl sulphoxide effects (DMSO) on experimental in vivo models of nociception and inflammation. Pharmacol Res. 2008;57:419-425.
33. Jacob SW, Herschler R. Pharmacology of DMSO. Cryobiology. 1986;23:14-27.
34. Adamson JE, Crawford HH, Horton CE. The action of dimethyl sulfoxide on the experimental pedicle flap. Surg Forum. 1966;17:491-492.
35. Kligman AM. Topical pharmacology and toxicology of dimethyl sulfoxide. JAMA. 1965;193:796-804.
36. Santos NC, Figueira-Coelho J, Martins-Silva J, Saldanha C. Multidisciplinary utilization of dimethyl sulfoxide: pharmacological, cellular, and molecular aspects. Biochem Pharmacol. 2003;65:1035-1041.
37. Trogan E, Feig JE, Dogan S, Rothblat GH, Angeli V, Tacke F, Randolph GJ, Fisher EA. Gene expression changes in foam cells and the role of chemokine receptor CCR7 during atherosclerosis regression in ApoE-deficient mice. Proc Natl Acad Sci U S A. 2006;103:3781-3786.
38 . Feig JE, Pineda-Torra I, Sanson M, Bradley MN, Vengrenyuk Y, Bogunovic D, Gautier EL, Rubinstein D, Hong C, Liu J, Wu C, van Rooijen N, Bhardwaj N, Garabedian M, Tontonoz P, Fisher EA. LXR promotes the maximal egress of monocyte-derived cells from mouse aortic plaques during atherosclerosis regression. J Clin Invest. 2010;120:4415-4424.
39. Verschuren L, De Vries-Van Der Weij J, Zadelaar S, Kleemann R, Kooistra T. LXR agonist suppresses atherosclerotic lesion growth and promotes lesion regression in apoE*3Leiden mice: time course and mechanisms. J Lipid Res. 2009;50:301-311.
40. Schreyer SA, Wilson DL, LeBoeuf RC. C57BL/6 mice fed high fat diets as models for diabetes-accelerated atherosclerosis. Atherosclerosis. 1998;136:17-24.
41. Paigen B, Mitchell D, Reue K, Morrow A, Lusis AJ, LeBoeuf RC. Ath-1, a gene determining atherosclerosis susceptibility and high density lipoprotein levels in mice. Proc Natl Acad Sci U S A. 1987;84:3763-3767.
42. Liao F, Andalibi A, deBeer FC, Fogelman AM, Lusis AJ. Genetic control of inflammatory gene induction and NF-kappa B-like transcription factor activation in response to an atherogenic diet in mice. J Clin Invest. 1993;91:2572-2579.
43. Johnston TP. The P-407-induced murine model of dose-controlled hyperlipidemia and atherosclerosis: a review of findings to date. J Cardiovasc Pharmacol. 2004;43:595-606.
44. Goldstein JL, Brown MS. The LDL receptor. Arterioscler Thromb Vasc Biol. 2009;29:431-438.
45. Van Craeyveld E, Gordts SC, Nefyodova E, Jacobs F, De Geest B. Regression and stabilization of advanced murine atherosclerotic lesions: a comparison of LDL lowering and HDL raising gene transfer strategies. J Mol Med. 2011; Epub ahead of print.
46. Kassim SH, Li H, Vandenberghe LH, Hinderer C, Bell P, Marchadier D, Wilson A, Cromley D, Redon V, Yu H, Wilson JM, Rader DJ. Gene therapy in a humanized mouse model of familial hypercholesterolemia leads to marked regression of atherosclerosis. PLoS One. 2010;5:e13424.
47. Yu H, Stoneman V, Clarke M, Figg N, Xin HB, Kotlikoff M, Littlewood T, Bennett M. Bone marrow-derived smooth muscle-like cells are infrequent in advanced primary atherosclerotic plaques but promote atherosclerosis. Arterioscler Thromb Vasc Biol. 2011;31:1291-1299.
48. Williams KJ, Feig JE, Fisher EA. Rapid regression of atherosclerosis: insights from the clinical and experimental literature. Nat Clin Pract Cardiovasc Med. 2008;5:91-102.
49. Ye D, Zhao Y, Hildebrand RB, Singaraja RR, Hayden MR, Van Berkel TJ, Van Eck M. The dynamics of macrophage infiltration into the arterial wall during atherosclerotic lesion development in low-density lipoprotein receptor knockout mice. Am J Pathol. 2011;178:413-422.

50. Potteaux S, Gautier EL, Hutchison SB, van Rooijen N, Rader DJ, Thomas MJ, Sorci-Thomas MG, Randolph GJ. Suppressed monocyte recruitment drives macrophage removal from atherosclerotic plaques of ApoE-/- mice during disease regression. J Clin Invest. 2011;121:2025-2036.