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Research paper

Dyslipidemia, steatohepatitis and atherogenesis in lipodystrophic apoE deficient mice with Seipin deletion

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ABSTRACT

SEIPIN is an integral membrane protein located in the endoplasmic reticulum, regulating adipocytes differentiation and lipolysis. Deficiency of Seipin in mice causes severe general lipodystrophy, accompanied by insulin resistance, postprandial hypertriglyceridemia and steatohepatitis. In atherosclerosis-prone Ldlr null ($Ldlr^{-/-}$) mice, lipodystrophy caused by Seipin deletion even led to severe hypercholesteremia and accelerated atherogenesis, when challenged with an atherogenic diet. However, whether the phenotypes observed in $Seipin^{-/-}Ldlr^{-/-}$ mice were a common consequence due to lipodystrophy, rather than genetic background restricted or diet dependent, was unknown. Herein we explored the lipodystrophy-related dyslipidemia, steatohepatitis and atherogenesis in another atherosclerosis-prone murine model, apolipoprotein E null ($apoE^{-/-}$) mice. Besides, we also compared phenotypes between sexes in $apoE^{-/-}$ mice with Seipin deletion ($Seipin^{-/-}apoE^{-/-}$). We found that compared with $apoE^{-/-}$ controls, $Seipin^{-/-}apoE^{-/-}$ mice also developed severe general lipodystrophy with hyperlipidemia, steatohepatitis and increased atherogenesis. Although the severity of adipose loss in female and male $Seipin^{-/-}apoE^{-/-}$ mice were similar, hyperlipidemia, steatohepatitis and atherosclerosis were less severe in females than in males. Therefore, we demonstrated that lipodystrophy-related metabolic disorders, caused by Seipin deletion, were independent of genetic background and experimental diet, as seen in $Ldlr^{-/-}$ and $apoE^{-/-}$ mice. However, gender factor affected the disease progression, with females more resistant to developing lipodystrophy-related metabolic consequences.

1. Introduction

Human lipodystrophy is a rare disease characterized by general or partial loss of adipose tissue. It's usually classified as congenital general lipodystrophy (CGL, also known as Berardinelli-Seip congenital lipodystrophy) or acquired lipodystrophy, based on the etiology. While the former CGL is caused by several genetic defects, the latter acquired lipodystrophy is more commonly seen in HIV-affected patients receiving antiretroviral therapy. Although these two types of lipodystrophy differ in origin and clinical manifestations, they share many metabolic consequences, such as insulin resistance, hypertriglyceridemia and steatohepatitis (Garg and Agarwal, 2009; Huang-Doran et al., 2010; Capeau et al., 2010).

Seipin is the culprit gene for the most severe form of human CGL, type 2 CGL. It encodes a homonymous integral membrane protein in the endoplasmic reticulum, which regulates adipocytes differentiation and lipolysis (Magre et al., 2001; Szymanski et al., 2007). Previously, we have generated mice deleted of Seipin (Cui et al., 2011). These $Seipin^{-/-}$ mice developed severe general lipodystrophy, followed by insulin resistance, postprandial hypertriglyceridemia and steatohepatitis, as seen in lipodystrophic patients with Seipin dysfunction, therefore represented a suitable disease model to study human lipodystrophy (Cui et al., 2011; Wang et al., 2015; Gao et al., 2015). To further explore the effects of lipodystrophy-related metabolic defects on cardiovascular

Abbreviations: CGL, congenital general lipodystrophy; Ldlr, low-density-lipoprotein receptor; apoE, apolipoprotein E; FPLC, fast protein liquid chromatography; WAT, white adipose tissue; BAT, brown adipose tissue; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoproteins; LDL, low-density lipoproteins; HDL, high-density lipoprotein

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Table 1
Analysis of adipose distribution in lipodystrophic apoE$^{-/-}$ mice with Seipin deletion.

	Female			Male		
	apoE$^{-/-}$	Seipin$^{-/-}$apoE$^{-/-}$	% loss of AT	apoE$^{-/-}$	Seipin$^{-/-}$apoE$^{-/-}$	% loss of AT
WAT (mg)						
Subcutaneous	267.9 ± 41.8	37.7 ± 5.9,**	85.9	362.2 ± 44.4	37.6 ± 4.5,***	89.6
Inguinal	43.2 ± 3.1	3.7 ± 1.1,***	91.4	62.0 ± 8.1	4.4 ± 1.6,***	92.9
Gonadal	257.7 ± 63.6	2.8 ± 1.5,*	98.9	605.6 ± 155.5	3.2 ± 1.6,**	99.5
Mesenteric	108.5 ± 33.7	26.4 ± 3.0, ns	75.6	246.1 ± 32.2	31.6 ± 5.9,***	87.2
Retroperitoneal	61.0 ± 18.1	0.0 ± 0.0,*	100.0	186.9 ± 37.4	0.0 ± 0.0,**	100.0
Total	738.1 ± 148.1	70.7 ± 9.0,**	90.4	1462.9 ± 265.6	76.8 ± 8.1,**	94.7
BAT (mg)						
Intrascapular	122.3 ± 12.0	71.3 ± 14.4,**	41.7	161.7 ± 17.1	59.3 ± 5.1,***	63.3

Data were presented as mean ± SEM.
AT: adipose tissue; WAT: white adipose tissue; BAT: brown adipose tissue.
ns: no significance.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

system, we crossed these Seipin$^{-/-}$ mice with low-density-lipoprotein receptor null (Ldlr$^{-/-}$) mice, a murine model of diet-induced atherosclerosis. We found that lipodystrophy caused by Seipin deletion even led to severe hypercholesteremia with accelerated atherogenesis, when challenged with an atherogenic high-cholesterol-high-fat diet (Wang et al., 2016). However, whether the phenotypes observed in Seipin$^{-/-}$Ldlr$^{-/-}$ mice were a common consequence due to lipodystrophy, rather than genetic background restricted or diet-dependent, was unknown. Herein we explored the lipodystrophy-related dyslipidemia, steatohepatitis and atherogenesis in another atherosclerosis-prone murine model, apolipoprotein E null (apoE$^{-/-}$) mice. Both Seipin$^{-/-}$apoE$^{-/-}$ mice and apoE$^{-/-}$ controls were fed rodent chow diet to 9 months, to explore the natural and long-term influence of lipodystrophy on metabolism without diet stress. Besides, we also compared phenotypes between two sexes in Seipin$^{-/-}$apoE$^{-/-}$ mice.

2. Materials and methods

2.1. Animals and diet

Seipin$^{-/-}$ mice were generated as previously described (Cui et al., 2011) and backcrossed with C57BL/6J mice for at least 8 generations. ApoE$^{-/-}$ mice on C57BL/6J background were provided by Peking University. Seipin$^{-/-}$ mice on C57BL/6J background were crossed with apoE$^{-/-}$ mice to generate Seipin$^{-/-}$apoE$^{-/-}$ mice. A total of 17 apoE$^{-/-}$ mice (including 9 females and 8 males) and 17 Seipin$^{-/-}$apoE$^{-/-}$ mice (including 10 females and 7 males) were included in the study. All mice were maintained on a 12-h-light/12-h-dark cycle with free access to water and normal rodent chow diet to 9 months old. All experiments in the study were following the NIH guidelines for the care and use of laboratory animals and approved by Animal Care Committee of Peking University Health Science Center.

2.2. Plasma lipids analysis

Mice were fasted for 4 h. Their blood was collected by retro-orbital venous plexus puncture and prepared as we previously described (Liao et al., 2015). Plasma total cholesterol and triglycerides were measured using commercial enzymatic kits (BioSino, Beijing, China). For lipoprotein distribution analysis, plasma samples pooled from 4 to 5 mice per group were applied to Tricorn high-performance Superose S-6 10/300 GL columns, fractioned by a fast protein liquid chromatography (FPLC) system (Amersham Biosciences, Buckinghamshire, UK) and followed by elution with PBS at a constant flow rate of 0.25 ml/min. Each eluted fraction was assayed for cholesterol and triglycerides concentrations using the commercial enzymatic kits described above.

2.3. Hepatic lipids analysis

Mice were euthanized and flushed with 20 ml 0.01M phosphate buffer solution through the left ventricle. The liver was removed and weighted.

For histological analysis of hepatic lipids, the liver was fixed in 4% paraformaldehyde for 20 min and later in 20% sucrose overnight. Then the liver was embedded in O.C.T. compound (Sakura Finetek, Torrance, USA), snap-frozen in liquid nitrogen and cross-sectioned at 7 μm thickness. Cryosections of the liver were stained with Oil-red O (counterstained with hematoxylin) to visualize hepatic lipid accumulation. Images were obtained with Leica graphic analysis system.

For quantitative analysis of hepatic lipid content, approximately 100 mg liver (wet weight) was weighed and homogenized in 1 ml PBS. Lipids were extracted using Folch's reagent, as we previously described (Wang et al., 2015), and dissolved in 1 ml 3% Triton X-100. Cholesterol and triglycerides contents in the solutions were measured with the enzymatic kits described above and then normalized to liver weight.

2.4. Atherosclerotic lesion analysis

Mice were euthanized and flushed as described above. The entire aorta and the heart were harvested and fixed as we previously described (Liao et al., 2017).The aorta was cut open longitudinally under a dissecting microscope after the adventitia was cleaned and then stained by Oil-red O (Sigma, St. Louis, USA). Images were obtained by a digital camera. Quantification was determined with Image J. The heart was embedded in O.C.T. compound, snap-frozen in liquid nitrogen and cross-sectioned serially at the aortic root level at 7 μm thickness. Cryosections of the aortic root were then stained with Oil-red O (counterstained with hematoxylin). Images were obtained with Leica graphic analysis system. Quantification was determined with Image J.

Macrophages in the atherosclerotic lesions were stained with rat-anti-mouse CD68 antibody (ab53444, diluted at 1:300; Abcam, Cambridge, UK). Briefly, cryosections of the aortic root were first incubated with 0.3% hydrogen peroxide for 10 min to block the endogenous peroxide activity, followed by blocking with 5% goat serum at 37 °C for 1 h, and then incubated with CD68 antibody at 4 °C overnight. Biotin was detected with DAB substrate kit (ZSJQ-Bio, Beijing, China). Images were obtained with Leica graphic analysis system. Quantification was determined with Image J.