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## REVIEW

### Roles of the Lipid Metabolism in Hepatic Stellate Cells Activation<sup>△</sup>

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**Abstract** The lipids present in hepatic stellate cells (HSCs) lipid droplets include retinyl ester, triglyceride, cholesteryl ester, cholesterol, phospholipids and free fatty acids. Activation of HSCs is crucial to the development of fibrosis in liver disease. During activation, HSCs transform into myofibroblasts with concomitant loss of their lipid droplets and production of excessive extracellular matrix. Release of lipid droplets containing retinyl esters and triglyceride is a defining feature of activated HSCs. Accumulating evidence supports the proposal that recovering the accumulation of lipids would inhibit the activation of HSCs. In healthy liver, quiescent HSCs store 80% of total liver retinols and release them depending on the extracellular retinol status. However, in injured liver activated HSCs lose their retinols and produce a considerable amount of extracellular matrix, subsequently leading to liver fibrosis. Further findings prove that lipid metabolism of HSCs is closely associated with its activation, yet relationship between activated HSCs and the lipid metabolism has remained mysterious.

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**T**HE lipids, such as retinyl ester, triglyceride, cholesteryl ester, cholesterol, phospholipids and free fatty acids, are present in hepatic stellate cells (HSCs) lipid droplets. <sup>1</sup> In liver injury and repair, activation of HSCs has been recognized as one of the first steps. The activated HSCs transform into myofibroblasts with concomitant loss of their lipid droplets and production of excessive extracellular matrix. <sup>2</sup> It is a characteristic point of HSCs activation that release of lipid

droplets, like retinyl esters and triglyceride. <sup>3</sup> Other evidence has shown that recovering the accumulation of lipids would inhibit HSCs activation. <sup>4</sup> Tang and Chen <sup>4</sup> reported that curcumin eliminated stimulatory effects of leptin on HSCs activation and increased adenosine monophosphate-activated protein kinase activity, leading to inducing expression of genes relevant to lipid accumulation and elevating the level of intracellular lipids. Lipid metabolism in HSCs has been reported to be closely associated with HSCs activation, yet relationship between HSCs activation and the lipid metabolism has remained unclear.

#### TRIGLYCERIDE METABOLISM AND HSCS ACTIVATION

The characteristic feature of a quiescent HSC is the

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presence of both vitamin A and triglycerides in the cytoplasm. HSCs activation is associated with depletion of the two lipid components.<sup>5</sup> Testerink *et al*<sup>2</sup> found that lipid droplet degradation in activated HSCs is a highly dynamic regulatory process. Activation of HSCs is crucial to the development of fibrosis in nonalcoholic fatty liver disease. Chen *et al*<sup>6</sup> revealed that liver fatty acid-binding protein, an abundant cytosolic protein that modulates fatty acid metabolism in enterocytes and hepatocytes, also modulates HSCs fatty acids utilization and in turn regulates the fibrogenic program. Thiazolidinediones markedly reduce hepatic steatosis in both rodents and humans. However, the effects and mechanisms of thiazolidinediones on hepatic fibrosis remain unclear. Thiazolidinediones has been found to improve hepatic fibrosis by activating the adenosine monophosphate-activated protein kinase signaling pathway in rats with nonalcoholic steatohepatitis.<sup>7</sup> High fat diet intake has been shown to activate HSCs in the rat's liver and induce collagen type I, transforming growth factor beta-1 and tumor necrosis factor expressions, which indicates high fat diet has the function of inducing the occurrence of hepatic fibrosis that is a pathological process associated with too much extracellular matrix deposition.<sup>8</sup> Nakano *et al*<sup>9</sup> investigated the preventive actions of bezafibrate against nonalcoholic steatohepatitis, the activation of HSCs, and fibrogenesis by using an *in vitro* model of nonalcoholic steatohepatitis. They found that bezafibrate improves hepatic steatosis and potentially prevents inflammation, oxidative stress, HSCs activation, and fibrogenesis in the liver.

The recovery of lipid deposition in HSCs has been proved to inhibit activation of HSCs.<sup>4</sup> Thus, transiting the activated HSCs back to the resting state, becomes a new strategy of treatment against liver fibrosis. Galli and Dubuquoy *et al*<sup>10,11</sup> found that the increase of lipid droplets in the cells transfected with peroxisome-proliferator activated receptor  $\gamma$  made the phenotype of HSCs re-fatted, and the HSCs transited from the active to the static.

### CHOLESTEROL METABOLISM AND HSCS ACTIVATION

Cholesterol metabolism disorders could have adverse effects on hepatic inflammation. The livers obtained from nonalcoholic, nonobese, non-insulin-resistant fatty liver disease rabbit model fed with food containing 1% cholesterol showed the physiopathological features of nonalcoholic fatty liver disease.<sup>12</sup> Carbon tetrachloride induced liver injury in apolipoprotein E-deficient (ApoE<sup>-/-</sup>) mice. Compared with wild-type mice, ApoE<sup>-/-</sup> mice exhibited

exacerbated hypercholesterolemia, higher hepatic transforming growth factor beta-1, and monocyte chemoattractant protein-1, and tissue inhibitor of metalloproteinase 1 expression levels, and enhanced hepatic oxidative stress. These findings manifest that hypercholesterolemic mice are susceptible to liver injury, because cholesterol causes liver inflammation and fibrosis.<sup>13</sup> Min *et al*<sup>14</sup> demonstrated that dysregulating cholesterol metabolism in nonalcoholic fatty liver disease may contribute to disease severity by evaluating the expression of cholesterol metabolic genes. Overexpression of transgenic cholesteryl ester hydrolase, which catalyzes the hydrolysis of stored intracellular cholesteryl esters, reduced cellular cholesterol overload in hepatic macrophages and improved hepatic insulin resistance and inflammation.<sup>15</sup>

The content of cholesterol in lysosome in Kupfer cells is an important factor for hepatic inflammation. Niemann-Pick type C disease is a lysosomal storage disease characterized by cholesterol and glycosphingolipids accumulation, and eventually leading to clinically hepatosplenomegaly and progressive neurodegeneration. The majority of patients die during their adolescence.<sup>16</sup> In addition, Bieghs *et al*<sup>17</sup> found a greater accumulation of cholesterol in lysosomes of Kupfer cells of Ldlr<sup>-/-</sup> mice (mice do not express the low density lipoprotein receptor) that received bone marrow transplants from Cyp27a1<sup>-/-</sup> mice (mice do not express the low density sterol 27-hydroxylase gene) after the high-cholesterol diet than those received bone marrow transplants from wild-type mice. Administration of 27-hydroxycholesterol to Ldlr<sup>-/-</sup> mice, following the high-cholesterol diet, reduced the accumulation of lysosomal cholesterol and hepatic inflammation, compared with the mice that were not given 27-hydroxycholesterol. So they proposed that accumulation of cholesterol in lysosomes of Kupfer cells might promote hepatic inflammation.

Though it is still not fully understood the contribution of cholesterol metabolic factors to the severity of liver disease, there are some interesting findings about it. Tokunaga *et al*<sup>18</sup> illustrated that if rats were fed with a synthetic cholesterol synthesis rate-limiting enzyme (HMG-CoA reductase), which was used to inhibit fluvastatin, for 2 days before 90% hepatectomy, the serum level of total bilirubin was observably lowered at 48 hours after hepatectomy, and the expression of alpha smooth-muscle actin protein in the liver was restrained. Furthermore, the liver regeneration improved significantly 72 hours after hepatectomy. Aprigliano *et al*<sup>19</sup> reported that, atorvastatin, a HMG-CoA reductase specific inhibitors, inducing apoptosis in activated HSCs, is related to an increased protease activity of

caspase-9 and -3. Through inhibiting the extracellular signal-regulated protein kinase1/2 (ERK1/2) activation by adding mitogen-activated protein kinase inhibitor U0126, atorvastatin-induced apoptosis could be blocked. Yang *et al*<sup>20</sup> detected cannabinoid receptor (CB1R and CB2R) in HSCs and HSCs growth could be concentration-dependent inhibited by anandamide, a kind of the endogenous cannabinoid N-long-chain fatty acid derivatives. Moreover, a high concentration of anandamide (20 micromol/L) triggers signal cell death but not apoptosis. By using cannabinoid receptor (CB1R and CB2R) antagonist, these effects could not be blocked, but through methyl-beta-cyclodextrin, a cholesterol depletory agent, the effects of cannabinoid receptor antagonist can be blocked. These results suggested that cannabinoid receptor (CB1R and CB2R) did not mediate the anandamide induced HSCs necrosis. Li *et al*<sup>21</sup> investigated the effect of transforming growth factor beta-1 on transcriptional repression of human cholesterol 7 alpha-hydroxylase gene (CYP7A1), they found that transforming growth factor beta-1 inhibited the mRNA expression of CYP7A1 in primary human liver cells and decreased bile acid synthesis in HepG2 cells.

### VITAMIN A METABOLISM AND HSCS ACTIVATION

Quiescent HSCs in healthy liver store 80% of total liver retinols and release them depending on the extracellular retinol status. However, HSCs activated by liver injury lose their retinols and produce a considerable amount of extracellular matrix, subsequently leading to liver fibrosis.<sup>22, 23</sup>

Mukhopadhyay *et al*<sup>24</sup> induced liver fibrosis in rat by bile duct ligation and administration of carbon tetrachloride (CCl<sub>4</sub>) respectively. The outcome indicated that vitamin A could ameliorate the consumption of lipid droplets in HSCs and lower expression of hepatic fibrotic markers such as anti-keratinocyte growth factor, anti-alpha-muscle actin, and anti-gliar fibrillary acidic protein antibodies. Testerink *et al*<sup>2</sup> revealed the rapid replacement of retinyl esters by polyunsaturating fatty acids in lipid droplets suggests a role of either lipids or their derivatives like eicosanoids in HSCs activation. He *et al*<sup>25</sup> found that all-trans retinoic acid alone or in combination with ursodeoxycholic acid substantially improved the growth rate of liver fibrosis in rats, reduced liver fibrosis and bile duct hyperplasia and lowered hepatic necrosis and liver hydroxyproline content obviously, moreover, downregulated the expression of liver transforming growth factor beta-1, collagen 1a1, matrix metalloproteinase 2, cytokeratin 19, anti-alpha-muscle actin, cytochrome P450 7A1, tumor necrosis factor alpha,

and interleukin-beta1.

When mice were fed with alcohol, its fatty liver formed through the activation of cannabinoid receptor-1 (CB1R), which increased lipogenesis and reduced fatty acid oxidation.<sup>26</sup> Mukhopadhyay *et al*<sup>24</sup> demonstrated that retinoic acid and its receptor agonists may regulate CB1R mRNA and protein expression and the retinoic acid receptor agonist CD437 and four hydrogen four methyl naphthalene acrylic acid (TTNPB) are the most effective regulators. Furthermore, the endo-cannabinoids 2-arachidonoylglycerol could increase the expression of hepatic CB1R.

In non-adipose cells, adipose differentiation-related protein (ADRP) regulates lipid droplet formation and lipolysis, so its function in HSCs attracts our attention. Lee *et al*<sup>26</sup> observed that both in liver fibrosis and culture-activated HSCs, ADRP was located in the lipid droplets. With liver fibrosis or cultured HSCs activation, lipid droplets disappeared and the expression of ADRP decreased. In the LX-2 human immortalized HSCs, retinol palmitate potentiated expression of LX-2 ADRP and improved generation of vitamin A palmitate and formation of lipid droplets. Inducing expression of ADRP can cause the decreased expression of anti-alpha-muscle actin mRNA and its protein. While ADRP gene was knockdowned with small interfering RNA, anti-alpha-muscle actin mRNA expressed normally. Vitamin A and palmitic acid induced expression of ADRP, which also led to decreasing expression of collagen type I and matrix metalloproteinase 2 in activated HSCs, while increasing expression of matrix metalloproteinase 1 mRNA and knockdown of human ADRP gene reversed these changes. Therefore, ADRP upregulation mediated by vitamin A and palmitic acid boosts downregulation of HSCs activation and functionally suppresses the expression of fibrogenic genes.

The phosphatidylinositol 3-kinase (PI3K) signaling pathway has been proved to regulate activation of HSCs, synthesis of collagen and cell proliferation. Son *et al*<sup>27</sup> has verified that inhibition of PI3K signaling pathway resulted in decreasing proliferation, reduced extracellular matrix deposition such as collagen type I, and restrained expression of fibrogenic genes.

In summary, HSCs lipid metabolism is closely associated with its activation, but relationship between the activation of HSCs and the lipid metabolism has remained mysterious.

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