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Commentary

Small-interfering RNA targeting proprotein convertase subtilisin/kexin type 9 might promote fatty liver disease and hepatocellular carcinoma through upregulation of CD36

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Abstract. Proprotein convertase subtilisin/kexin type 9 (PCSK9) binds to low-density lipoprotein (LDL) receptor and fatty acid translocase CD36, inducing lysosomal degradation of these two receptors in the liver cells. Both monoclonal antibody (mAb) and small-interfering RNA (siRNA) targeting PCSK9 have been designed for treatment of familial hypercholesterolemia recently, with elevating LDL receptors on the liver cell surface and increasing LDL uptake as the main beneficial mechanism. However, given that the binding domains of PCSK9 for LDL receptor and CD36 are different, and PCSK9 mAb only attacks the domain for LDL receptor, CD36 expression remains partially controlled under PCSK9 mAb treatment. In contrast, PCSK9 siRNA brings on complete loss of PCSK9, resulting in overexpression of CD36. Based on the fact that CD36 is a key factor in the pathogenesis of non-alcoholic fatty liver disease (NAFLD) and subsequent hepatocellular carcinoma (HCC), the risk of developing NAFLD and HCC on long-term use of PCSK9 siRNA is thus raised as a hypothesis. Additionally, because CD36 is also involved in the promotion of malignant diseases other than HCC, such as acute myeloid leukemia, gastric cancer, breast cancer, and colorectal cancer, the speculative danger of flourishing these malignancies by PCSK9 siRNA is discussed as well.

Keywords: Fatty acid translocase CD36, hepatocellular carcinoma, non-alcoholic fatty liver disease, proprotein convertase subtilisin/kexin type 9, small-interfering RNA

1. Introduction

Non-alcoholic fatty liver disease (NAFLD), histologically ranging from non-alcoholic fatty liver to non-alcoholic steatohepatitis (NASH), has evolved to be the most common cause of cryptogenic cirrhosis and one of the main preceding etiologies for development of hepatocellular carcinoma (HCC) [1]. Meta-analysis studies had shown that NAFLD significantly increased the risk of HCC [2], with a

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38% prevalence of HCC in non-cirrhotic NASH, much higher than that in non-cirrhotic liver diseases of other etiologies [3]. Treatment of important predisposing factors for NAFLD, such as diabetes, obesity, and metabolic syndrome, has been aggressively advocated to diminish the threat of NAFLD but survey of HCC in NAFLD still remains a challenge because HCC could develop in both cirrhotic and non-cirrhotic NAFLD, while the latter traditionally has not been a focus of HCC screening [4]. Thus, it seems crucial that any possible strategies should be adopted to avoid risky issues linked to the development of NAFLD and subsequent HCC.

Importantly, free fatty acids have been found to be closely related to progressive liver fibrosis seen in NAFLD with their ability to trigger the profibrogenic Hippo signaling transcriptional coactivator yes-associated protein 1 through p38 mitogen-activated protein kinase pathway [5]. Based on the recognition of transmembrane fatty acid translocase cluster of differentiation 36 (CD36) as a master in regulating cellular lipid metabolism by facilitating cellular uptake of long-chain free fatty acids [6], herein, the potential pathogenic roles of CD36 in NAFLD and HCC will be reviewed and two kinds of lipid-lowering agents, the monoclonal antibody (mAb) and small-interfering RNA (siRNA) against proprotein convertase subtilisin/kexin type 9 (PCSK9) which regulates CD36 expression, will be examined to see whether these modern therapeutic modalities for hypercholesterolemia might have adverse effects on liver regarding the risk of NAFLD and HCC.

2. CD36 in the pathogenesis of NAFLD and consecutive HCC

CD36 is a multiligand transmembrane receptor which can bind thrombospondin-1, thrombospondin-2, long chain fatty acid, oxidized low density lipoprotein, and oxidized phospholipids, acting also as a receptor for pathogen-associated molecular patterns in activation of innate immunity and clearance of cell debris with phagocytosis besides its role in fatty acid transportation [7]. As a fatty acid translocase, the hydrophobic extracellular domains of CD36 enable fatty acids to be translocated through cell membrane into cells with a high efficiency of fatty acid utilization such as adipose tissue, skeletal muscle, heart, and liver [8].

It has been demonstrated in cultured hepatoma cells that transport of fatty acid into cells is promoted by formation of a heterotetrameric protein complex comprising CD36, caveolin-1, fatty acid-binding protein, and calcium-independent membrane phospholipase A2 [9]. Notably, fatty acid itself can increase CD36 expression via stimulating transcription factor SOX2 [10], then take advantage of CD36-mediated oxidative stress to make hepatocytes activated and involved in the process of liver fibrosis [11]. Therefore, CD36 is considered to be the key driver of fatty acid-related lipotoxicity, including elevated endoplasmic reticulum stress, reactive oxygen species, insulin resistance, tissue damage, apoptosis, and impaired autophagy, during the process of NAFLD with modulation of its expression in the liver as a major influencing factor for the progression of hepatosteatosis [12].

In addition to its pathological role in NAFLD, overexpression of CD36 was detected in human HCC, in two research works being considered to cause the development of HCC. One of those studies discovered that the highly upregulated CD36 exerted a stimulatory effect on HCC growth and metastasis through activating the Src/PI3K/AKT/mTOR signaling pathway-dependent aerobic glycolysis [13]. The other showed that the overexpressed CD36 was able to accelerate the progression of HCC by promoting the expression of aldo–keto reductases family 1 member C2 and increasing fatty acids uptake [14]. Furthermore, it has been demonstrated in murine and human NAFLD-associated HCC tissues that CD36-mediated oxidized low-density lipoprotein (LDL) uptake can activate CCAAT/enhancer-binding protein beta expression to straightly upregulate Nogo-B, a metabolic modulator which interacts with autophagy-related 5 to enhance yes-associated protein 1 oncogenic activity, escalating high-fat diet-induced metabolic disturbance and carcinogenesis in the liver [15].

Even more interestingly, epithelial-mesenchymal transition, a well-known characteristic of cancer progression, has been revealed to be induced by CD36-mediated upward free fatty acid intake in HCC cells via the TGF-β and Wnt/β-catenin signaling pathways [16]. Beside fatty acids, CD36 also cooperates with cartilage oligomeric matrix protein secreted by hepatic stellate cells to boost proliferation, invasion, and metastasis of HCC cells by activating MEK/ERK and PI3K/AKT signaling pathways [17]. Hence, it is no wonder why CD36 has been chosen as an emerging therapeutic target for HCC given its pivotal role in metabolic dysregulation involved in tumour initiation and progression [18].

3. Add-on use of PCSK9 mAb and siRNA in lipid control

Hypercholesterolemia is associated with the pathogenesis of a variety of disorders, inclusive of atherosclerotic cardiovascular disease, NAFLD, obesity, diabetes, neurodegenerative diseases, cancer, osteoporosis, and virus infection. Many novel therapeutic modalities aiming at different targets to lower cholesterol levels have been innovated recently. Among those targets, PCSK9 emerges as a striking one, especially for familial hypercholesterolemia [19].

PCSK9, which belongs to the proprotein convertase family and consists of three domains (pro-domain, catalytic domain, and Cys-His-rich domain) in its mature form. It is synthesized in hepatocytes and pancreatic β-cells. PCSK9 can bind to LDL receptor intracellularly within Golgi apparatus or extracellularly on the cell surface when released into plasma. Consequently it induces lysosomal degradation of LDL receptor, reducing uptake of cholesterol to the cell, and ultimately leading to elevated cholesterol levels in the blood. The very-low density lipoprotein receptor is also a target of degradation by PCSK9. Thus, gain-of-function mutations in PCSK9 gene have been identified to be the causes of familial hypercholesterolemia while loss-of-function mutations in PCSK9 gene resulted in hypocholesterolemia [20]. Innovative strategies in development for blocking PCSK9 function and alleviating hypercholesterolemia include mAb, siRNA, vaccine, clustered regularly interspaced short palindromic repeats (CRISPR)/Cas system of gene deletion/editing, and orally active inhibitors. Anti-PCSK9 mAb blocks the interaction between PCSK9 and LDL receptor, reduces the degradation of LDL receptor, and consequently lowers LDL levels. Vaccines induce antibodies targeting PCSK9. Orally active inhibitors are small molecule LDL receptor analogs which can also interfere with the binding of PCSK9 to LDL receptor. siRNA acts by sequences-specific degradation of PCSK9 mRNA and functionally inhibits PCSK9 gene expression. CRISPR/Cas system decreases PCSK9 levels by inducing DNA double-strand breaks, resulting in error structures in PCSK9 gene [21]. So far, major beneficial cholesterol homeostasis outcomes have been detected in clinical trials using mAbs and siRNAs against PCSK9 [22]. These outcomes have received supportive confirmation from meta-analysis studies [23, 24], prompting recommendations for add-on use to achieve better lipid control and prevent cardiovascular diseases in patients already on other lipid-lowering agents, still the treatment goals remain unmet [25].

4. A speculation based on different influence of PCSK9 mAb and siRNA on CD36 expression

PCSK9 not only can bind to LDL receptor but also to CD36 in adipocytes and liver cells, subsequently giving rise to degradation of CD36 and lowering cellular uptake of long-chain fatty acids [26]. Nonetheless, the PCSK9 domains which attach to LDL receptor and CD36 are not the same. Degradation of LDL receptor requires the catalytic unit of PCSK9, while the C-terminal Cys-His rich domain (CHRD) of PCSK9 is responsible for CD36 binding. In the presence of PCSK9 mAbs, which attack PCSK9 on the catalytic domain and rescue LDL receptor from endocytosis, the C-terminal CHRD of

PCSK9 can still tie up CD36 and bring on partial reduction of CD36 through endosomes/lysosomes degradation [27]. On the contrary, silencing PCSK9 synthesis with siRNA decreases the whole PCSK9 product and presumably preserves the LDL receptor and CD36 on the cell surface [28].

On that account, a meta-analysis of clinical trials designed for treating hypercholesterolemia with siRNA targeting PCSK9 (inclisiran) revealed a 51% decrease of LDL level and a 24% lower major adverse cardiovascular events rate as compared with placebo [29]. Additionally, a pooled patient-level analysis of inclisiran trials for familial hypercholesterolemia disclosed a 50.7% placebo-corrected drop of LDL levels [30]. However, the risk of persistent elevated CD36 expression in hepatocytes on long-term use of PCSK9 siRNA ought to be meticulously examined.

Animal experiments demonstrated clearly that high fat-diet was able to induce hepatic endoplasmic reticulum stress, insulin resistance, NASH, and fibrosis in PCSK9 knockout mice [31]. Moreover, when injected with the hepatic carcinogen diethylnitrosamine early-in-life, PCSK9 knockout mice were found to develop liver cancer more likely than wild type mice [32]. Accordingly, based on the pathogenic roles of CD36 in liver diseases, it seems reasonable to assume that deletion of PCSK9 expression for a long duration via PCSK9 siRNA treatment might promote the development of NAFLD or even HCC due to uncontrolled CD36 expression.

5. Discussion

Intriguingly, single neucleotide polymorphism of PCSK9 gene with nonsynoymous variants in the population has been detected years ago. As we know from published reports that both heterozygous and homozygous carriers of PCSK9 R46 L variant had comparatively lower lipoprotein(a) and LDL cholesterol levels than non-carriers, resulting in a significantly reduced odds ratio for myocardial infarction or aortic stenosis, presumably due to decreased atherosclerotic burden [33]. This finding was confirmed by a series of population studies and meta-analyses [34]. Whether this particular variant leads to hepatosteatosis, however, is a problem of debate, with positive and negative opinions [35, 36]. Importantly, the so-called loss-of-function of this variant probably interferes with the regulation of LDL receptor expression only. The R46 L missense mutation locates at the pro-domain, which remains associated with the catalytic domain responsible for LDL receptor binding after maturation of PCSK9 [37]. Therefore, it likely does not affect the CHRD responsible for CD36 binding and degradation. It is no wonder then that the R46 L variant, assumed here to still be capable of downregulating liver cell surface CD36, was shown to be protective against liver damage in people with NAFLD [38]. On the other hand, the missense mutation in the so-called gain-of-function *PCSK9* variant (rs505151 E670 G) locating at the CHRD [37], probably increases CD36 binding to a great extent, leading to downregulation of CD36 and the consequent lipid profile abnormality [39]. In other words, let's consider the possibility that the gain-of-function of E670 G actually targets at CD36 rather than LDL receptor. If this hypothesis was true, carriers of E670 G variant would have lower incidence of NAFLD. All these speculations, nevertheless, await evidence support from further laboratory works and epidemiologic studies.

Just like its engagement in the pathogenesis of HCC, CD36 upregulation has been detected in other malignant tumors with fatty acids and a variety of other ligands as CD36 activators in accelerating tumor initiation, proliferation, invasion, metastasis, immunosuppression, chemoresistance, and radioresistance [40]. For instance, in acute myeloid leukemia, CD36-overexpressed leukemia stem cells were peculiarly capable of proliferation [41], which was probably stimulated by apolipoprotein C2 through CD36-mediated LYN–ERK signaling activation [42]. In gastric cancer cell lines, higher CD36 expression led to more aggressive characteristics of migration, invasion, epithelial-mesenchymal transition, and fatty acid-mediated metastasis through the AKT/GSK-3β/β-catenin signaling pathway [43, 44].

Similar results of enhanced epithelial-mesenchymal transition and migration potential generated by fatty acids from cancer-associated fibroblasts via CD36 were seen in mouse models of colorectal cancer [45], and a conceivable mechanism was disclosed to be upregulation of the metallopeptidase MMP28 tightly connected to the overexpression of CD36 in colorectal cancer cells [46]. In breast cancer, CD36-mediated fatty acid uptake not only could potentiate invasiveness of cancer cells [47], but also foster the resistance to HER2-targeted therapies [48]. As a consequence, CD36 has been classified as a prospective therapeutic target for various types of cancer [49].

When taken together, the risk of developing NAFLD, HCC, and a lot of other malignant tumors on long-term eradication of PCSK9 with ensuing CD36 overexpression looks not an exaggerated warning. Although no significant difference of adverse effects was observed between siRNA targeting PCSK9 (inclisiran) and placebo in three major phase 3 clinical trials for management of familial hypercholesterolemia, the observation time is up to 540 days only [50], which is much shorter than what is presumed for a carcinogenic process. Even more worrisome is a recent proposal that people without familial hypercholesterolemia could start on lipid-lowering therapy plus inclisiran as well at thirty years of age, to reduce the cumulative LDL and atherosclerotic plaque burden afterwards [51]. In my opinion, the danger of developing NAFLD, HCC, and many other cancers on totally obliterating PCSK9 should firstly be excluded by regularly scheduled laboratory and image studies during follow-up in carefully designed clinical trials lasting for at least ten years before raising such an assertion, especially when more novel gene silencing therapies targeting PCSK9 are coming [52].

Finally, the atypical antipsychotic drug clozapine had been revealed to be capable of increasing CD36 expression in macrophages through a NADPH oxidase-reactive oxygen species-peroxisome proliferator-activated receptor γ pathway [53]. Coincidentally, this drug has been disclosed to be able to worsen glucose intolerance and NAFLD in obese mice [54], with the fact that schizophrenia patients on clozapine have a remarkable 43.2% prevalence of metabolic syndrome, of which NAFLD is a component [55]. Additionally, in two large epidemiologic researches, clozapine has recently found to be significantly, in a dose-response manner associated with hematological malignancies, that is, lymphoma and leukemia, as been compared with non-use of clozapine [56, 57]. These astonishing findings remind us again of the possible adverse effects of CD36 overexpression resulting from genetic abolishing of PCSK9. Thus, it is ardently hoped that more intensive investigations could be performed to study the safety of siRNA and other gene silencing therapies targeting PCSK9 in the near future.

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Conflict of interest

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