The Regulation of Proprotein Convertase Subtilisin/Kexin Type 9 and Its Liver Involvement

R. G. Mihăilă¹,²*

¹Faculty of Medicine, Lucian Blaga University of Sibiu, 500169 Sibiu, Romania.
²Department of Hematology, Emergency County Clinical Hospital, Sibiu, Romania.

Author’s contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

ABSTRACT

Introduction: Anti-proprotein convertase subtilisin/kexin type 9 (PCSK9) antibodies have been very effective to lower low-density lipoprotein (LDL)-cholesterol. They attracted the attention on PCSK9 enzyme role in multiple pathways and underlined the complex correlations between lipid metabolism and various other liver or extrahepatic diseases, that are insufficiently known. Hepatocyte nuclear factor 1, sterol regulatory element-binding protein (SREBP) 1c and SREBP2 are the main modulators of liver PCSK9 gene and protein expression, processes that can also be influenced by some natural and synthetic compounds (as berberine, or bortezomib and rosuvastatin, respectively), endoplasmic reticulum stress, metabolic status and the diurnal pattern. Aim: This minireview is an analysis of PCSK9 involvement in liver pathology. Results and Conclusion: PCSK9 is a key enzyme which increases LDL-receptor degradation. Hepatitis C virus (HCV) enters into hepatocytes in combination with lipoproteins through LDL-receptors and negatively modulates the PCSK9 expression to reduce LDL-receptor degradation and increase HCV entry into hepatocyte. PCSK9 modulates the hepatic CD81 - a mediator of post-attachment process. The greater the liver lipid accumulation the higher the plasma levels of PCSK9, which were observed in non-alcoholic fatty liver disease. A decreased expression of PCSK9 and an increased expression of LDL-receptor were shown in liver samples from patients with non-alcoholic fatty liver disease. 

*Corresponding author: E-mail: romeomihaila@yahoo.com;
with hepatocellular carcinoma, a fact that suggests that these cancer cells are able to modulate their local microenvironment to obtain a higher amount of cellular cholesterol. With better understanding of the role of this enzyme, PCSK9 and the factors involved in its regulation can become targets for the treatment of different liver pathologies.

Keywords: Hepatitis C; hepatocellular carcinoma; LDL-receptor; non-alcoholic fatty liver disease; proprotein convertase subtilisin/kexin type 9.

1. INTRODUCTION

The discovery of direct acting antivirals (DAA) against hepatitis C constitutes a turning point in hepatology. Their ability to cure 90% of patients is undoubtedly a success. But worrisome information concerning the high rate of relapse of hepatocellular carcinoma (HCC) appeared recently: after treatment with DAA, 27.6% of 58 patients with prior HCC developed radiologic liver cancer recurrence after a median monitoring period of 5.7 months [1]. In another study, 17 of 59 patients treated with DAA developed HCC recurrences during a 24-week follow-up period. In addition, 3.16% of patients diagnosed of having chronic hepatitis C, later developed HCC [2]. An explanation of tumor recurrence or occurrence could be the next: the liver inflammatory infiltrate appeared as response to chronic HCV infection includes also immune competent cells that early recognize tumor transformed cells and removes them; the quick disappearance of viral infection and liver inflammatory infiltrate creates a local immune deficiency, which can contribute to tumor relapse. But is this the only explanation for tumor relapse? HCV is able to negatively modulate proprotein convertase subtilisin/kexin type 9 (PCSK9) expression, to reduce low-density lipoprotein receptor (LDL-receptor) degradation and increase cholesterol and HCV entry into hepatocyte [3]. It is known that cholesterol is essential for the metabolism of malignant cells and it has been recently found that PCSK9 has a decreased expression in HCC [4]. This entry of a high amount of cholesterol into the hepatocyte could be an important step in HCC development and the local immune deficiency explains the lack of recognition and removal of recently appeared tumor cells. But such an increase of cholesterol entry into hepatocyte could also be found in patients with severe hypercholesterolemia treated with monoclonal antibodies against PCSK9. It would be useful to know whether these patients are prone to develop hepatocellular carcinoma. It is obvious that there are correlations between lipid metabolism and various liver diseases that are still not fully understood and known. Therefore, I decided to write a minireview collecting data in this field existing in PubMed since January 2014, using the terms “PCSK9 regulation”, “PCSK9 review”, and "liver".

PCSK9 was previously known as, or called neural apoptosis-regulated convertase 1 (NARC - 1) and its expression was found not only in hepatocytes, but also in some epithelial cells (from intestine and colon), mesenchymal cells (from kidney) and brain telencephalen neurons [5,6]. PCSK9 is synthesized in an inactive form - a zymogen; proprotein convertases remove a section of peptide chains of PCSK9 structure and activates it [7]. PCSK9 is not involved in in LDL-receptor reduction, but potentially enhances the LDL-receptor intracellularly degradation after the binding to epidermal growth factor like domain repeat A (EGFA) of the extracellular portion of LDL-receptor and endocytosis of so formed complex; a pH under 5.5 significantly enhances the PCSK9 binding affinity [6,8]. The role of secreted PCSK9 protein is to contribute to cellular and liver LDL-receptor degradation, followed by an increase of LDL-cholesterol serum levels in vivo [6]. Liver PCSK9 expression produces a significant decrease of VLDL-receptor in adipose tissue, but a higher cell surface VLDL-receptor expression in fat tissue and increased adipose mass were shown in PCSK9 deficient mice [6,9]. The average plasma level of PCSK9 in humans is estimated around 100-500 ng/mL and is significantly associated with LDL-cholesterol and total cholesterol [6,10]. Despite its role in cholesterol metabolism, PCSK9 only explains about 7% of the variability in plasma LDL-cholesterol levels [6,11]. PCSK9 is also associated with plasma triglycerides, as a result of its possible role in VLDL metabolism [6].

2. KEY ELEMENTS INVOLVED IN THE LIVER METABOLISM OF LIPIDS

2.1 PCSK9 and LDL-receptor

The complex lipid metabolism occurs with liver involvement. Liver LDL-receptor facilitates
cholesterol penetration into hepatocyte. Its regulation has a double regulation: at both transcriptional and post-transcriptional level. PCSK9 is a key enzyme involved in an increased LDL-receptor degradation. But liver cholesterol content is the result of the action of many factors, including those involved in transcription, as: sterol regulatory element binding protein (SREBP) and liver X receptor [12]. In addition, between hepatic pathology and lipid metabolism may exist interactions, which are being increasingly studied.

PCSK9 is synthesized by the liver [13,14], circulates through the bloodstream [14], and has the role to bind to the LDL-receptors [13]. This leads to decreased availability of LDL-receptors [15]. After endocytosis, the enzyme bound to LDL-receptor is involved in an increased endosomal/lysosomal degradation [13,16] of the complex consisting of receptor and the bound LDL-cholesterol [13]. The result is the inhibition of LDL (including cholesterol) uptake [17]. In this way, it intervenes in the reduction of plasma LDL-cholesterol clearance. PCSK9 diminishes also liver regeneration by the decreased cholesterol uptake [4]. The presence of a loss-of-function mutation in PCSK9 gene results in a significant reduction of plasma LDL-cholesterol levels. This finding has led to anti-PCSK9 monoclonal antibodies development. They absorb PCSK9 and allow the LDL-receptor to dissociate from LDL-cholesterol and recycle. Thus, the density of LDL-receptor increases at the same time with the LDL-cholesterol clearance [13]. Therefore, anti-PCSK9 monoclonal antibodies are also involved in the regulation of LDL metabolism [18].

The liver expression of LDL-receptor depends not only on the activity of PCSK9, but also on the presence of aprotein E. The serum cholesterol level could not be reduced with anti-PCSK9 antibody in apoprotein E-deficient mice [19].

2.2 The Regulation of PCSK9 on the Transcriptional Levels

It is known that an upregulation of liver PCSK9 protein causes a higher LDL-receptor degradation, followed by a decreased uptake of apoB lipoproteins and a consequent elevation of their plasma levels, including those of LDL and chylomicron remnants [20]. Some liver transcription factors are involved in lipid metabolism regulation. While SREBP-1c independently regulates especially the synthesis of fatty acids, SREBP2 controls the synthesis of cholesterol, but also that of PCSK9 and LDL receptor [21].

In addition, hepatocyte nuclear factor (HNF) 1α and 1β are positive regulators of liver PCSK9 transcription in hamster species. It links to a site embedded in the proximal region of PCSK9 promoter. A liver-specific knockdown of either HNF1α or HNF1β could antagonize the rosuvastatin-induced increase of serum PCSK9 level and, in this way, contributed to the decrease of serum cholesterol level in normolipidemic hamsters [22]. But HNF1α and not HNF1β represents the primary positive regulator of PCSK9 transcription at least in the mice's liver [23]. Moreover, it is a key transactivator for PCSK9 gene expression [24]. The mutation of the HNF1 site was able to reduce PCSK9 promoter activity with over 90% and attenuate the action of nuclear SREBP2 to transactivate PCSK9 promoter in HepG2 cells [25].

Berberine is a natural compound with a hypocholesterolemic effect which is able to contribute to an accelerated degradation of HNF1α protein and, subsequently, to a reduction of HNF1α-mediated PCSK9 gene transcription in HepG2 cells [24]. It has been found that a HNF1 binding site resides 28 bp upstream from sterol response elements and is important for PCSK9 transcription and regulation in HepG2 cells [25]. However, berberine produces not only a modest reduction of HNF1α, but also of nuclear SREBP2; the result is an important suppression of PCSK9 transcription via these two critical regulatory sequences; thus, SREBP pairs with HNF1 to control PCSK9 transcription, a process involved in the control of cholesterol metabolism [25]. This effect of berberine can be eradicated using bortezomib – a proteasome inhibitor, which increases HNF1α and PCSK9 cellular levels [24].

Rosuvastatin induces two transactivators of PCSK9 transcription (HNF1α and SREBP2) and only one involved in LDL-receptor transcription. This explains the result consisting of a predominant effect of PCSK9 in LDL-receptor degradation in the hamster liver [26].
Endoplasmic reticulum (ER) stress can activate SREBP2, a transcription factor localized in ER and involved in PCSK9 up-regulation, as it has been stated above. ER Ca2+ depletion promotes the process of SREBP2 activation and further PCSK9 transcription. Instead, any factor that produces ER stress independent of its ability to dysregulate ER Ca2+ inhibits PCSK9 secretion (which remains in ER), thus reducing PCSK9-mediated LDL-receptor degradation [27].

How can the metabolic status and the diurnal pattern influence PCSK9 gene and protein expression? In the fasting state, PCSK9 plasma levels are reduced through modulation of HNF1α, SREBP1c and SREBP2. They are also positively associated with insulinemia and insulin resistance. Their regulation through SREBP1c is independent of glucose status. Dietary intake of n-3 polyunsaturated fatty acids are involved in the reduction of PCSK9 plasma concentration and liver PCSK9 mRNA expression, while fructose intake seems to upregulate PCSK9 mRNA expression and PCSK9 plasma levels [20].

2.3 The Link between PCSK9 and Triglyceride Metabolism

PCSK9 stimulates intestinal production of triglyceride-rich apolipoprotein B (apoB) lipoproteins by a transcriptional increase of apoB and contributes to an augmentation of apoB protein stability via both LDL-receptor dependent and LDL-receptor independent pathways [28]. PCSK9 is also involved in an increased liver lipid and lipoprotein production through apoE- and LDL-receptor dependent pathways. Human PCSK9 was found in the artery wall and directly influences atherosclerosis lesion size and structure dependently on LDL-receptor and independently of plasma lipid and lipoprotein modifications [29].

PCSK9 modulates (reduces) the function of CD36 (an important receptor which plays a role in long-chain fatty acids transport and triglyceride storage) and triglyceride metabolism, independent of its action on LDL-receptor. PCSK9 limits the fatty acid uptake and triglyceride storage in some tissues (as the liver) by CD36 degradation [30]. The relationship between PCSK9 and CD36 requires further investigation in order to appreciate its practical importance.

3. THE COMPLEX REGULATION OF PCSK9

3.1 The Role of Sterol Regulatory Element Binding Protein-2 (SREBP-2) and SREBP Cleavage-activating Protein (SCAP)

While PCSK9 is involved in posttranscriptional downregulation of LDL-receptor expression, SREBP-2 and SCAP intervene in transcriptional tightly regulation of the receptor (they promote the transcription of LDL-receptor gene) [31]. SREBPs activation is dependent on site-1 protease (S1P), which is a key enzyme. As was pointed, SREBPs is involved in PCSK9 upregulation, which influences the level of LDL-receptor expression. In this way, liver S1P is a modulator of plasma apoB-containing lipoprotein [32]. But LDL-receptor pathway could be disrupted in various situations (hyperglycemia, renin-angiotensin system activation, or inflammation) and is involved in different organ injuries caused by lipid disorders, as non-alcoholic fatty liver disease or various locations of atherosclerosis. Another mechanism involved in upregulation of LDL-receptor expression at both transcriptional and post-transcriptional level is the mechanistic target of rapamycin (mTOR) complex 1 activation, which conduces to lipid deposition (Fig. 1) [31].

3.2 Indol Liver Expression

Another mechanism involved in PCSK9 blood levels augmentation is represented by liver indol overexpression, which acts through SREBP-2 and LDL-receptor pathway [33].

3.3 The Role of Sortilin

The mechanism of action of PCSK9 is not completely known. In this regard, the positive influence of sortilin – a high-affinity sorting receptor for PCSK9, codified by sortilin 1 (SORT1) gene, helps to clarify an issue. Sortilin facilitates PCSK9 secretion from primary hepatocytes. Its diminished or overexpression in the liver can modify PCSK9 plasma levels in humans (it makes them lower and higher respectively) [34].

3.4 The Circadian Regulation

The genes involved in liver cholesterol metabolism have also a circadian regulation. Two distinct groups of genes intervene in this scope during light-dark cycles: some of them
manifest a rhythmic expression pattern and the others – a non-rhythmic one. A disruption of the PCSK9/LDL-receptor regulatory axis was observed after a liver-specific inactivation of BMAL1. Tribbles homolog 1 (TRIB1) has a non-rhythmically expression. The liver clock ablation disturbs diurnal regulation of genes involved in hepatic lipid metabolism and TRIB1 gene. Experimental induction of TRIB1 gene in a mice liver lacking a functional liver clock was able to diminish plasma PCSK9 protein levels and increase LDL-receptor expression [35].

3.5 Experimental Results

There are peculiarities on lipid metabolism depending on the type of animal, and the data obtained from them cannot be extrapolated to humans. A high fructose diet given to hamsters produced a liver decrease and a plasma increase of PCSK9, while hepatic LDL-receptor protein levels diminished. The majority of hamster plasma PCSK9 was active concerning its ability to promote liver LDL-receptor degradation in vitro. In contrast, differences were found in mice feed with the same diet: the level of PCSK9 decreased both in plasma and liver, while hepatic LDL-receptor protein levels did not diminish [36]. The activation of PCSK9 gene in mice occurs through the binding of transcription factors transcription factor HNF1α and not 1β on its site located on the promoter region of PCSK9 gene [37]. It was shown that a high fat diet given to rats produced higher plasma LDL-cholesterol levels, but did not modify the plasma PCSK9 level and the liver LDL-receptor expression [38]. A pathological overexpression of matrix metalloproteinase-2 in Hepa1-c1c7 cells may protect the LDL-receptor against PCSK9-induced degradation [39]. An experimental model of hypercholesterolemia induced in wild-type mice used an adeno-associated virus to introduce a human D374Y gain-of-function mutant form of PCSK9. A synergy was found between this type of enzyme and apoprotein E deficiency [40]. The LXR-regulated E3 ubiquitin ligase inducible degrader of the LDL-receptor (IDOL) is involved in the control of LDL-receptor stability, process which is not dependent on SREBP or PCSK9. Liver X receptor (LXR) activation induces liver IDOL expression, diminishes LDL-receptor protein levels, and increases plasma LDL levels in cynomolgus monkeys. LXR agonist does not modify neither the liver IDOL transcript levels nor plasma LDL levels in mice. IDOL inhibition could be a way to LDL reduction in human beings [41]. The increased degradation of the LDL-receptor induced by PCSK9 does not depend on sortilin or amyloid precursor-like protein 2 in a mice model and ex vivo [42].

3.6 The Role of MicroRNAs

MiR-27a induces a 3-fold increase of PCSK9 and binds to 3’ untranslated region of LDL-receptor gene. In this way, it contributes to a reduction by 40% of LDL-receptor levels. MiR-27a is also involved in low-density lipoprotein receptor-related protein 6 (LRP6) and low density lipoprotein receptor adaptor protein 1 (LDLRAP1) decrease - lipoprotein receptors needful for an efficient liver endocytosis of the complex between LDL-receptor and LDL-cholesterol. Lock nucleic acids could be used to inhibit miR-27a and lower cholesterol levels by double mechanism (through the action on LDL-receptor and PCSK9) [43].

4. ANTI-PCSK9 MONOCLONAL ANTIBODIES AND OTHER MODALITIES TO REDUCE HYPERCHOLESTEROLEMIA

Not only are PCSK9 inhibitors very effective to lower LDL-cholesterol, but it seems that they do not cause severe liver toxic effects, in contrast with other lipid-lowering agents [44]. Indeed, anti-PCSK9 antibodies were safe and well-tolerated, according to a recent large meta-analysis. In addition, evolocumab was able to decrease the rate of abnormal hepatic function [45]. But a possible risk of their use could be the achievement of subphysiological plasma LDL-cholesterol values. Some programs initiated to monitor the potential risk of such low values were developed [46].

Statins contribute to an increasing of LDL-receptor level [43], but also of PCSK9 [43,47], which has an opposite effect on the level of LDL-receptors [43]. For example, pravastatin produces an upregulation of PCSK9, but its combination with MG132, a specific proteasome inhibitor, conduces to an increased LDL-receptor expression and LDL uptake in HepG2 cells, while the upregulation of PCSK9 was blocked [48]. The increase of PCSK9 induced by statins limits their effectiveness as hypocholesterolemic drugs [47]. It is accepted today that polymorphisms in various proteins can be involved in the resistance to statins; the following are among them: PCSK9, apolipoprotein E, LDL-receptor [49].
Loss-of-function PCSK9 mutations coexist with low LDL-cholesterol values. Such a loss-of-function mutation was realized applying clustered regularly interspaced short palindromic repeats. These were able to reduce cholesterolemia in mice and may be one future way to reduce serum cholesterol level in humans [50].

Ezetimibe, a hypocholesterolemic drug which diminishes cholesterol absorption in the small intestine, also produces an increase of PCSK9, LDL-receptor, SREBP2 and HNF-1α expression in the rat liver. The higher PCSK9 expression is accomplished by the SREBP2 and HNF-1α pathways [51].

Single domain antibodies are an alternative to the use of monoclonal antibodies. Their production is easier and cheaper. There are four single domain antibodies which recognize the C-terminal Cys-His-rich domain of PCSK9. They do not disturb the binding of PCSK9 to the LDL-receptor, but rather block the cellular LDL-receptor degradation [52].

**Fig. 1. The regulatory mechanism of PCSK9 and LDL-R in the liver**
5. OTHER DRUGS THAT INFLUENCE PCSK9 METABOLISM

Cholesteryl ester transfer protein (CETP) is involved in the transfer of cholesteryl ester from high-density lipoproteins (HDL) to very low- and low-density lipoproteins. The result is an increase of HDL-cholesterol and a decrease of LDL-cholesterol. Some studies made in vitro and in vivo established that CETP inhibitors produced a decrease of the mature form of SREBP2, responsible for a reduced transcription of liver PCSK9 and LDL-receptor [53]. PCSK9 can also influence the metabolism of triglyceride-rich lipoproteins. Plasma PCSK9 levels are correlated with triglyceride levels and some markers of carbohydrate metabolism. PCSK9 can influence postprandial lipemia and the liver apolipoprotein B production, and fibrate administration can influence plasma PCSK9 levels [54]. Berberine, a salt of benzylisoquinoline alkaloid with cholesterol-lowering properties, inhibits PCSK9 transcription mediated by HNF1α, through an increased HNF1α degradation in HepG2 cells. Bortezomib, an ubiquitin proteasome system inhibitor, increased in a dose-dependent manner the HNF1α protein content in HepG2 cells. In this way, bortezomib contributed to an increase of HNF1α and PCSK9 cellular levels, while LDL-receptor protein decreased. It eradicated the berberine effect on HNF1α and PCSK9 gene transcription process [55]. A diminished expression of PCSK9 and of other key genes involved in cholesterol and lipid metabolism was obtained in vitro and in vivo (in mouse liver) using 5-azacytidine, a DNA-hypomethylating agent [56], utilized in the treatment of myelodysplastic syndrome and acute myeloid leukemia. This drug inhibits de novo pyrimidine synthesis, followed by a disruption of lipid and cholesterol metabolism [56]. MG132, another proteasome inhibitor, can suppress PCSK9 expression in the HepG2 cells in a time-dependent manner, via a SREBP-1c related mechanism. The result is a dose-dependent increase of both LDL-receptor mRNA and protein levels and hepatocyte LDL uptake in a short-term treatment and of LDL-receptor protein in a long-term treatment [48]. The molecules involved in PCSK9 expression are presented in Table 1.

6. PCSK9 IN LIVER DISEASES

6.1 Hepatitis C Virus (HCV) Infection

There are connections between the presence of HCV into hepatocyte and lipid metabolism, which started to be better known. HCV acts to increase the intracellular lipid content, which is used for its own replication. HCV enters into hepatocytes in combination with lipoproteins through LDL-receptors. LDL-receptor expression is stimulated by HCV both in hepatocellular carcinoma Huh7 cells and liver tissue fragments collected from patients diagnosed with chronic hepatitis C. The viral regulation of LDL-receptor expression take place both at transcriptional and posttranslational level and occurs in infected hepatocytes. The transcription process is stimulated by SREBPs, which have an essential role. The PCSK9 expression is negatively modulated by HCV to reduce LDL-receptor degradation and increase HCV entry into hepatocyte [3].

Therefore, HCV enters into hepatocyte using LDL-receptor. There was suspicion on the possibility that some forms of PCSK9 could reduce the surface expression of CD81, another important component of HCV hepatocyte entry complex (as in the case of an artificial non-secreted, cell membrane-bound form of antigen), but it was shown that alirocumab (a monoclonal antibody anti-PCSK9) did not modify the expression levels of CD81. Thus, the susceptibility to HCV entry in human hepatocyte Huh-7 cells did not increase [15]. The combination of HCV with lipoproteins (lipoviral particles) can contribute to an increased virus penetration into hepatocyte. This process involves an attachment of heparan sulphate proteoglycans and LDL-receptor, followed by the action of CD81, which is a mediator of postattachment process. PCSK9 intervenes not only in LDL-receptor increased degradation, but also in the modulation of hepatic CD81 levels. An inverse correlation between lipoviral particles and apoprotein E was found in HCV genotype 3 and a reverse situation in HCV genotype 1, fact that implies a possible different apoprotein E mediation of viral entry into hepatocytes, dependent on the virus genotype. The plasma PCSK9 levels were lower in the genotype 3 vs genotype 1 of HCV. The diminished levels of PCSK9 and LDL in patients with genotype 3 involves an increased LDL-receptor activity [57]. The relationship between PCSK9 and CD81 requires further investigation in order to appreciate its practical importance.

6.2 Non-alcoholic Fatty Liver Disease

A disruption of cholesterol metabolism can be involved in the progression of non-alcoholic fatty liver disease augmented by inflammation.
Table 1. The role of different molecules in PCSK9 expression

| Molecule                                      | Effect on PCSK9                                                                                                                                                                                                 | Reference |
|-----------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| HCV                                           | Negatively modulates PCSK9 expression (in order to reduce LDL-receptor degradation and increase HCV entry into hepatocyte)                                                                                  | [3]       |
| Genotype 3 / genotype 1 of HCV                | Lower blood PCSK9 levels in genotype 3 vs genotype 1 of HCV                                                                                                                                               | [57]      |
| Mouse models of hepatic S1P knockdown through activation of the SREBPs | Decreases the liver and blood levels of the PCSK9                                                                                                                                                         | [32]      |
| Liver indol overexpression, which acts through SREBP-2 and LDL-receptor pathway               | Increases the blood levels of PCSK9                                                                                                                                                                        | [33]      |
| Sortilin decrease or overexpression           | Decreases or, respectively, increases plasma PCSK9 levels                                                                                                                                                 | [34]      |
| Induction of Trib1 gene in a mice liver lacking a functional liver clock                      | Decreases plasma PCSK9 levels                                                                                                                                                                             | [35]      |
| High amount of fructose in hamsters diet      | Produces liver decrease and a serum increase of PCSK9                                                                                                                                                     | [36]      |
| High amount of fructose in mice diet          | Decreases PCSK9 both in serum and liver Activates PCSK9 gene                                                                                                                                              | [36]      |
| Transcription factors hepatic nuclear factor 1α | Increases PCSK9                                                                                                                                                                                             | [37]      |
| MiR-27a                                       | Increase PCSK9                                                                                                                                                                                                | [43]      |
| Statins                                       | Blocks the upregulation of PCSK9                                                                                                                                                                            | [48]      |
| The combination of pravastatin with MG132 - a specific proteasome inhibitor                 | Increase PCSK9                                                                                                                                                                                               | [43,47]   |
| Ezetimibe                                      | Increase of PCSK9                                                                                                                                                                                             | [51]      |
| Single domain antibodies against PCSK9        | Recognize the C-terminal Cys-His-rich domain of PCSK9 and block the cellular LDL-receptor degradation                                                                                                       | [52]      |
| Berberine                                      | Inhibits PCSK9 transcription mediated by HNF1α, through an increased HNF1α degradation in HepG2 cells                                                                                                        | [55]      |
| Bortezomib                                     | Increases HNF1α and PCSK9 cellular levels                                                                                                                                                                  | [55]      |
| 5-azacytidine gived in vitro and in vivo mouse liver | Diminishes the expression of PCSK9                                                                                                                                                                          | [56]      |
| MG132 - a proteasome inhibitor - gived in HepG2 cells                                      | Can suppress PCSK9 expression                                                                                                                                                                              | [48]      |

in mice. An increased activity of mammalian target of rapamycin complex 1 contributes to this progression by a disruption of LDL-receptor expression through transcriptional and posttranscriptional pathways [58]. The greater the liver lipid accumulation the higher the plasma levels of PCSK9. It was even observed a correlation between plasma PCSK9 levels and the presence of liver steatosis and an association between this enzyme and an activation of lipogenesis. The modulation of this enzyme synthesis and release could be involved in non-alcoholic fatty liver disease (NAFLD) pathway [17], an exciting area that have to be studied further.

### 6.3 Hepatocellular Carcinoma

In primary rat hepatocytes and rat hepatoma cells insulin was able to rise the level of PCSK9 expression and LDL-receptor degradation in a PCSK9-dependent way, but insulin is not the most important regulator of PCSK9 [59]. The immunohistochemistry study of tissue obtained from 39 patients with hepatocellular carcinoma showed a decreased expression of PCSK9 and an increased expression of LDL-receptor, fact that suggests that these cancer cells are able to modulate their local microenvironment to obtain a higher amount of cellular cholesterol - a constant energy supply. The authors raise the question of
whether PCSK9 could be a target for hepatocellular carcinoma therapy [4]. But we could also raise the issue if the therapy with anti-PCSK9 could help the hepatocellular carcinoma emergence (at least in some patient populations), although there is still no data in the literature in this regard.

6.4 Liver Cirrhosis
A study made on noncholestatic cirrhotic patients who received a liver graft from primary deceased-donor established the importance of cholesterol availability for graft survival. Thus, a low pre-transplant serum cholesterol level in cirrhotic patients and an improper graft post-reperfusion response to hypocholesterolemia expressed through the inability to reduce the PCSK9 / LDL-receptor ratio were causes of graft loss [60].

6.5 Liver Lipid Clearance
The lipid clearance which takes place in the liver, via the mechanism PCSK9 – LDL-receptor, includes that of lipid pathogens, such as lipopolysaccharide. A decreased PCSK9 activity in human liver was found to be associated with a raised pathogen lipid clearance through LDL-receptor, a diminished inflammatory reaction, and better septic shock evolution [61].

7. CONCLUSION
PCSK9 is a key enzyme involved in LDL-receptor degradation. SREBP-2 and SCAP intervene in transcriptional tightly upregulation of PCSK9 [23], but the pathway could be disrupted in various situations.

The increase of PCSK9 induced by statins limits their effectiveness as hypocholesterolemic drugs [47]. Ezetimibe produces also an increase of PCSK9, LDL-receptor, SREBP2 and HNF-1α expression in the rat liver [51]. Anti-PCSK9 antibodies have been safe and well-tolerated in studies done until now, but they should be watched for possible adverse effects still unknown and on the achievement of subphysiological plasma LDL-cholesterol values [46].

The PCSK9 expression is negatively modulated by HCV to reduce LDL-receptor degradation and increase HCV entry into hepatocyte [3]. PCSK9 intervenes also in the modulation of hepatic CD81 levels [57], an important component of HCV hepatocyte entry complex [15]. The diminished levels of PCSK9 and LDL in patients with genotype 3 involves an increased LDL-receptor activity [57].

The greater the liver lipid accumulation the higher the plasma levels of PCSK9 which were observed in non-alcoholic fatty liver disease [17].

A decreased expression of PCSK9 and an increased expression of LDL-receptor were shown by immunohistochemistry in liver samples from patients with hepatocellular carcinoma, suggesting that these cancer cells are able to modulate their local microenvironment to obtain a higher amount of cellular cholesterol [4].

CONSENT
It is not applicable.

ETHICAL APPROVAL
It is not applicable.

COMPETING INTERESTS
Author has declared that no competing interests exist.

REFERENCES
1. Reig M, Mariño Z, Perelló C, Iñarrairaegui M, Ribeiro A, Lens S, et al. Unexpected early tumor recurrence in patients with hepatitis C virus-related hepatocellular carcinoma undergoing interferon-free therapy: A note of caution. J Hepatol. 2016;65:719-26.
2. Conti F, Buonfiglioli F, Scuteri A, Crespi C, Bolondi L, Caraceni P, et al. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. J Hepatol. 2016;65(4):727-33.
3. Syed GH, Tang H, Khan M, Hassanein T, Liu J, Siddiqui A. Hepatitis C virus stimulates low-density lipoprotein receptor expression to facilitate viral propagation. J Virol. 2014;88(5):2519-29.
4. Bhat M, Skill N, Marcus V, Deschenes M, Tan X, Bouteaud J, et al. Decreased PCSK9 expression in human hepatocellular carcinoma. BMC Gastroenterol. 2015;15:176.
5. Seidah NG, Benjannet S, Wickham L, Marcinkiewicz J, Jasmin SB, Stifani S, et al. The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): Liver regeneration and neuronal differentiation. Proc Natl Acad Sci USA. 2003;100:928-33.
6. Cao G, Konrad RJ, Kowala MC, Wang J. Novel regulators of low-density lipoprotein receptor and circulating LDL-C for the prevention and treatment of coronary artery disease. In: Angelo Squeri Ed. Coronary artery disease - New insights and novel approaches, InTech; 2012. Available:http://www.intechopen.com/books/coronary-artery-disease-new-insights-and-novel-approaches/novel-regulators-of-low-density-lipoprotein-receptor-and-circulating-ldl-c-for-the-prevention-and-treatment-of-coronary-artery-disease (Accessed September 09, 2016)

7. Lagace TA. PCSK9 and LDLR degradation: Regulatory mechanisms in circulation and in cells. Curr Opin Lipidol. 2014;25(5):387-93.

8. Zhang DW, Lagace TA, Garuti R, Zhao Z, McDonald M, Horton JD, et al. Binding of PCSK9 to EGF-A repeat of LDL receptor decreases receptor recycling and increases degradation. J Biol Chem. 2007;282:18602-12.

9. Roubtsova A, Munkonda MN, Awan Z, Marcinkiewicz J, Chamberland A, Lazure C, et al. Circulating proprotein convertase subtilisin/kexin 9 (PCSK9) regulates VLDLR protein and triglyceride accumulation in visceral adipose tissue. Arterioscler Thromb Vasc Biol. 2011;31:785-91.

10. Konrad RJ, Troutt JS, Cao G. Effects of currently prescribed LDL-C-lowering drugs on PCSK9 and implications for the next generation of PCSK9-lowering agents. Lipids Health Dis. 2011;10:38.

11. Lakoski SG, Lagace TA, Cohen JC, Horton JD, Hobbs HH. Genetic and metabolic determinants of plasma PCSK9 levels. J Clin Endocrinol Metab. 2009;94:2537-43.

12. Ramasamy I. Recent advances in physiological lipoprotein metabolism. Clin Chem Lab Med. 2014;52(12):1695-727.

13. Richter K, Barthel A, Bornstein SR, El-Armouche A, Wagner M. PCSK9 inhibitors - the magic bullet for LDL cholesterol reduction? Dtsch Med Wochenschr. 2016;141(12):863-9.

14. Marais AD, Kim JB, Wasserman SM, Lambert G. PCSK9 inhibition in LDL cholesterol reduction: Genetics and therapeutic implications of very low plasma lipoprotein levels. Pharmacol Ther. 2015;145:58-66.

15. Ramanathan A, Gusrrova V, Stahl N, Gurnett-Bander A, Kyratsous CA. Alirocumab, a therapeutic human antibody to PCSK9, does not affect CD81 levels or hepatitis C virus entry and replication into hepatocytes. PLoS One. 2016;11(4):e0154498.

16. Roubtsova A, Chamberland A, Marcinkiewicz J, Essalmani R, Fazel A, Bergeron JJ, et al. PCSK9 deficiency unmasks a sex- and tissue-specific subcellular distribution of the LDL and VLDL receptors in mice. J Lipid Res. 2015;56(11):2133-42.

17. Ruscica M, Ferri N, Macchi C, Meroni M, Lanti C, Ricci C, et al. Liver fat accumulation is associated with circulating PCSK9. Ann Med. 2016;48:384-91.

18. Sahebkar A, Chew GT, Watts GF. Recent advances in pharmacotherapy for hypertriglyceridemia. Prog Lipid Res. 2014;56:47-66.

19. Ason B, van der Hoorn JW, Chan J, Lee E, Pieterman EJ, Nguyen KK, et al. PCSK9 inhibition fails to alter hepatic LDLR, circulating cholesterol, and atherosclerosis in the absence of ApoE. J Lipid Res. 2014;55(11):2370-9.

20. Krysa JA, Ooi TC, Proctor SD, Vine DF. Nutritional and lipid modulation of PCSK9: Effects on cardiometabolic risk factors. J Nutr. 2017;147(4):473-81.

21. Rong S, Cortés VA, Rashid S, Anderson NN, McDonald JG, Liang G, et al. Expression of SREBP-1c requires SREBP-2-mediated generation of a sterol ligand for LXR in livers of mice. Elife. 2017;6 pii: e25015.

22. Dong B, Singh AB, Shende VR, Liu J. Hepatic HNF1 transcription factors control the induction of PCSK9 mediated by rosuvastatin in normolipidemic hamsters. Int J Mol Med. 2017;39(3):749-56.

23. Shende VR, Wu M, Singh AB, Dong B, Kan CF, Liu J. Reduction of circulating PCSK9 and LDL-C levels by liver-specific knockdown of HNF1α in normolipidemic mice. J Lipid Res. 2015;56(4):801-9.

24. Dong B, Li H, Singh AB, Cao A, Liu J. Inhibition of PCSK9 transcription by berberine involves down-regulation of hepatic HNF1α protein expression through the ubiquitin-proteasome degradation pathway. J Biol Chem. 2015;290(7):4047-58.

25. Li H, Dong B, Park SW, Lee HS, Chen W, Liu J. Hepatocyte nuclear factor 1alpha plays a critical role in PCSK9 gene transcription and regulation by the natural
hypocholesterolemic compound berberine. J Biol Chem. 2009;284(42):28885-95.

26. Dong B, Wu M, Li H, Kraemer FB, Adeli K, Seidah NG, et al. Strong induction of PCSK9 gene expression through HNF1a and SREBP2: Mechanism for the resistance to LDL-cholesterol lowering effect of statins in dyslipidemic hamsters. J Lipid Res. 2010;51(6):1486-95.

27. Lebeau P, Al-Hashimi A, Sood S, Lhoták Š, Yu P, Gyulay G, et al. Endoplasmic Reticulum Stress and Ca2+ Depletion Differentially Modulate the Sterol Regulatory Protein PCSK9 to Control Lipid Metabolism. J Biol Chem. 2017;292(4):1510-23.

28. Rashid S, Tavori H, Brown PE, Linton MF, He J, Giunzioni I, et al. Proprotein convertase subtilisin kexin type 9 promotes intestinal overproduction of triglyceride-rich apolipoprotein B lipoproteins through both low-density lipoprotein receptor-dependent and -independent mechanisms. Circulation. 2014;130(5):431-41.

29. Tavori H, Giunzioni I, Predazzi IM, Plubell D, Shivinsky A, Miles J, et al. Human PCSK9 promotes hepatic lipogenesis and atherosclerosis development via apoE- and LDLR-mediated mechanisms. Cardiovasc Res. 2016;110(2):268-78.

30. Demers A, Samami S, Lauzier B, Des Rosiers C, Sock ET, Ong H, et al. PCSK9 induces CD36 degradation and affects long-chain fatty acid uptake and triglyceride metabolism in adipocytes and in mouse liver. Arterioscler Thromb Vasc Biol. 2015;35(12):2517-25.

31. Zhang Y, Ma KL, Ruan XZ, Liu BC. Dysregulation of the low-density lipoprotein receptor pathway is involved in lipid disorder-mediated organ injury. Int J Biol Sci. 2016;12(5):569-79.

32. Basu D, Huq A, Iqbal J, Hussain MM, Jiang XC, Jin W. Hepatic S1P deficiency lowers plasma cholesterol levels in apolipoprotein B-containing lipoproteins when LDLR function is compromised. Nutr Metab (Lond). 2015;12:35.

33. Sasaki M, Teray O, Ayaori M, Uto-Kondo H, lizuka M, Yogo M, et al. Hepatic overexpression of idol increases circulating protein convertase subtilisin/kexin type 9 in mice and hamsters via dual mechanisms: sterol regulatory element-binding protein 2 and low-density lipoprotein receptor-dependent pathways. Arterioscler Thromb Vasc Biol. 2014;34(6):1171-8.

34. Gustafsen C, Kjolby M, Nyegaard M, Mattheisen M, Lundhede J, Buttenschon H, et al. The hypercholesterolemia-risk gene SORT1 facilitates PCSK9 secretion. Cell Metab. 2014;19(2):310-8.

35. Ma D, Liu T, Chang L, Rui C, Xiao Y, Li S, et al. The liver clock controls cholesterol homeostasis through Trib1 protein-mediated regulation of PCSK9/low density lipoprotein receptor (LDLR) axis. J Biol Chem. 2015;290(52):31003-12.

36. Dong B, Singh AB, Azhar S, Seidah NG, Liu J. High-fructose feeding promotes accelerated degradation of hepatic LDL receptor and hypercholesterolemia in hamsters via elevated circulating PCSK9 levels. Atherosclerosis. 2015;239(2):364-74.

37. Shende VR, Wu M, Singh AB, Dong B, Kan CF, Liu J. Reduction of circulating PCSK9 and LDL-C levels by liver-specific knockdown of HNF1a in normolipidemic mice. J Lipid Res. 2015;56(4):801-19.

38. Jia YJ, Liu J, Guo YL, Xu RX, Sun J, Li JJ. Dyslipidemia in rat fed with high-fat diet is not associated with PCSK9-LDL-receptor pathway but ageing. J Geriatr Cardiol. 2013;10(4):361-8.

39. Wang X, Berry E, Hernandez-Anzaldo S, Sun D, Adijiang A, Li L, et al. MMP-2 inhibits PCSK9-induced degradation of the LDL receptor in Hepa1-c1c7 cells. FEBS Lett. 2015;589(4):490-6.

40. Roche-Molina M, Sanz-Rosa D, Cruz FM, García-Prieto J, López S, Abia R, et al. Induction of sustained hypercholesterolemia by single adeno-associated virus-mediated gene transfer of mutant hPCSK9. Arterioscler Thromb Vasc Biol. 2015;35(1):50-9.

41. Hong C, Marshall SM, McDaniel AL, Graham M, Layne JD, Cai L, et al. The LXR-Idol axis differentially regulates plasma LDL levels in primates and mice. Cell Metab. 2014;20(5):910-8.

42. Butkinaree C, Canuel M, Essalmani R, Poirier S, Benjannet S, Asselin MC, et al. Amyloid precursor-like protein 2 and sortilin do not regulate the PCSK9 convertase-mediated low density lipoprotein receptor degradation but interact with each other. J Biol Chem. 2015;290(30):18609-20.

43. Alvarez ML, Khosroheidari M, Eddy E, Done SC. MicroRNA-27a decreases the level and efficiency of the LDL receptor and contributes to the dysregulation of
cholesterol homeostasis. Atherosclerosis. 2015;242(2):595-604.

44. Sarges P, Steinberg JM, Lewis JH. Drug-induced liver injury: Highlights from a review of the 2015 literature. Drug Saf. 2016;39:801-21.

45. Zhang XL, Zhu QQ, Zhu L, Chen JZ, Chen QH, Li GN, et al. Safety and efficacy of anti-PCSK9 antibodies: A meta-analysis of 25 randomized, controlled trials. BMC Med. 2015;13:123.

46. Olsson A. PCSK9 inhibition - A new era in cholesterol treatment. Lakartidningen. 2015;112.

47. Norata GD, Tibolla G, Catapano AL. PCSK9 inhibition for the treatment of hypercholesterolemia: Promises and emerging challenges. Vascul Pharmacol. 2014;62(2):103-11.

48. Yan H, Ma YL, Gui YZ, Wang SM, Wang XB, Gao F, et al. MG132, a proteasome inhibitor, enhances LDL uptake in HepG2 cells in vitro by regulating LDLR and PCSK9 expression. Acta Pharmacol Sin. 2014;35(8):994-1004.

49. Reiner Z. Resistance and intolerance to statins. Nutr Metab Cardiovasc Dis. 2014;24(10):1057-66.

50. Ding Q, Strong A, Patel KM, Ng SL, Gosis BS, Regan SN, et al. Permanent alteration of PCSK9 with in vivo CRISPR-Cas9 genome editing. Circ Res. 2014;115(5):488-92.

51. Xu RX, Liu J, Li XL, Li S, Zhang Y, Jia YJ, et al. Impacts of ezetimibe on PCSK9 in rats: study on the expression in different organs and the potential mechanisms. J Transl Med. 2015;13:87.

52. Weider E, Susan-Resiga D, Essalmani R, Hamelin J, Asselin MC, Ashraf Y, et al. Proprotein convertase subtilisin/Kexin type 9 (PCSK9) single domain antibodies are potent inhibitors of LDL receptor degradation. J Biol Chem. 2016;291:16659-71.

53. Dong B, Singh AB, Fung C, Kan K, Liu J. CETP inhibitors downregulate hepatic LDL receptor and PCSK9 expression in vitro and in vivo through a SREBP2 dependent mechanism. Atherosclerosis. 2014;235(2):449-62.

54. Druce I, Abujrud H, Ooi TC. PCSK9 and triglyceride-rich lipoprotein metabolism. J Biomed Res. 2015;29:429-36.

55. Dong B, Li H, Singh AB, Cao A, Liu J. Inhibition of PCSK9 transcription by berberine involves down-regulation of hepatic HNF1α protein expression through the ubiquitin-proteasome degradation pathway. J Biol Chem. 2015;290(7):4047-58.

56. Poirier S, Samami S, Mamarbachi M, Demers A, Chang TY, Vance DE, et al. The epigenetic drug 5-azacytidine interferes with cholesterol and lipid metabolism. J Biol Chem. 2014;289(27):18736-51.

57. Bridge SH, Sheridan DA, Felmslee DJ, Crosseey MM, Fenwick Fl, Lanyon CV, et al. PCSK9, apolipoprotein E and lipoviral particles in chronic hepatitis C genotype 3: evidence for genotype-specific regulation of lipoprotein metabolism. J Hepatol. 2015;62(4):763-70.

58. Liu J, Ma KL, Zhang Y, Wu Y, Hu ZB, Lv LL, et al. Activation of mTORC1 disrupted LDL receptor pathway: A potential new mechanism for the progression of non-alcoholic fatty liver disease. Int J Biochem Cell Biol. 2015;61:8-19.

59. Miao J, Manthena PV, Haas ME, Ling AV, Shin DJ, Graham MJ, et al. Role of insulin in the regulation of proprotein convertase subtilisin/kexin type 9. Arterioscler Thromb Vasc Biol. 2015;35(7):1589-96.

60. Ginianni Corradini S, Siciliano M, Parlati L, Molinaro A, Cantafora A, Poli E, et al. Recipient perioperative cholesterolaemia and graft cholesterol metabolism gene expression predict liver transplant outcome. Liver Int. 2014;34(7):e290-301.

61. Walley KR, Thain KR, Russell JA, Reilly MP, Meyer NJ, Ferguson JF, et al. PCSK9 is a critical regulator of the innate immune response and septic shock outcome. Sci Transl Med. 2014;6(258):258ra143.