Review

PCSK9 inhibition in the management of familial hypercholesterolemia

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A R T I C L E   I N F O

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A B S T R A C T

Familial hypercholesterolemia (FH) is a frequent hereditary metabolic disease characterized by high serum low-density lipoprotein (LDL) cholesterol concentration and premature atherosclerotic cardiovascular disease (ASCVD). The discovery of the LDL receptor as one of the causative genes of FH enabled us to understand the pathophysiology of FH and paved the way for developing statins. Similar to LDL receptor, discovery of proprotein convertase subtilisin/kexin type 9 (PCSK9) also created an opportunity for developing its inhibitors. Since PCSK9 degrades LDL receptor protein, inhibiting PCSK9 will be an effective strategy. Evolocumab and alirocumab, anti-PCSK9 antibodies that inhibit binding between PCSK9 and LDL receptors, are now available in Japan. Adding an anti-PCSK9 antibody to standard therapy with statin alone or statin combined with ezetimibe further reduced serum LDL cholesterol levels by around 60% and they significantly decrease cardiovascular event incidence as compared with placebo. Additionally, the strong LDL cholesterol lowering effect of anti-PCSK9 antibody therapies has reportedly enabled the frequency of lipoprotein apheresis to be reduced or to be discontinued. As alternative strategies against PCSK9, antisense oligonucleotide agents that inhibit PCSK9 protein synthesis as well as a small interfering (or short interference) RNA (siRNA) for PCSK9 are also being developed. While relatively high cost can be given as a problem, PCSK9 inhibitors are able to reduce LDL cholesterol dramatically even in FH patients who could not achieve targets until now. To ensure that these drugs are given to the patients who really need them, it is necessary to raise the diagnosis rate and family screening has to be more actively conducted. Finally, it has been reported that PCSK9 is expressed not only in hepatocytes but also in other cells such as epithelial cells in small intestine and vascular smooth muscle cells in atherosclerotic plaque. Further research regarding extra-hepatic pathophysiology of PCSK9 is expected.

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Introduction

Familial hypercholesterolemia (FH) is an autosomal dominant hereditary metabolic disease characterized by markedly high serum low-density lipoprotein cholesterol (LDL-C) concentration, xanthomas including Achilles tendon thickening and premature coronary artery disease (CAD). In FH patients, as the prevalence of CAD is extremely high and its age of onset is 15–20 years earlier than usual, early diagnosis and appropriate treatment to prevent atherosclerosis or delay its progression is necessary. Various studies have reported high rates for FH heterozygotes of 1 in 200–500 persons and that 10% of patients with acute coronary syndrome have FH. Thus, from the aspect of public health, it can be said that FH is one of the most important underlying diseases of CAD in Japan.

The discovery of the LDL receptor as one of the causative genes of FH enabled us to understand the pathophysiology of FH and paved the way for developing 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins). Similar to LDL receptor, discovery of proprotein convertase subtilisin/kexin type 9 (PCSK9) also created an opportunity for developing its inhibitors. Thus, we have to recognize that patients with single gene disorder such as FH have given some clues to develop new pharmacological interventions that reduce LDL-C (Table 1). This review focuses on PCSK9, a novel therapeutic target for dyslipidemia and atherosclerosis. In it, we outline the pathologies arising from genetic abnormalities in PCSK9 as well as the new drugs that have been developed based on clues gained from such pathologies, and finally look at the issues in the treatment of FH and its future prospects.

Discovery of PCSK9 and description of its physiological actions

In 2003, Abifadel et al. [1] found that gene mutations in the PCSK9 genes were shown to cause dominant hypercholesterolemia in pedigree analysis, and also demonstrated that a gain of function due to a missense mutation in this gene was the cause of this disease.

To date, a number of gain of function mutations associated with hypercholesterolemia and premature atherosclerotic cardiovascular disease (ASCVD) have been identified [2]. Among Japanese patients with FH, it has been reported that a PCSK9 E32K variant affects LDL-C levels and could exacerbate the clinical phenotype of heterozygous FH carrying 3 types of LDL receptor mutations [3]. We reported that the additional PCSK9 V41 variant carrying LDL receptor mutations was linked to a significantly increased prevalence of ASCVD in accord with the elevation of the LDL-C level [4].

We also found that the prevalence of PCSK9 gain of function variants such as V41 and E32K was 9% in the patients without LDL receptor mutations [4]. Patients carrying only PCSK9 V41 or E32K variants were 2.7% and 5.8%, respectively, of the 224 unrelated FH patients. Interestingly, the distribution of PCSK9 gain of function variants among Japanese heterozygous FH patients is different from that in previous reports of PCSK9 gain of function mutations (S127R, D129G, F216L, R218S, R357H, and D374H) in FH studies from other countries. In a French population, the reported prevalence of FH patients carrying only PCSK9 gain of function variants without LDL receptor mutations was 0.7% [5]. The prevalence of PCSK9 gain of function variants among Japanese FH patients may be higher than that in FH patients from other countries.

Two years after the identification of gain of function mutations, PCSK9 loss of function mutations were reported to be associated with lower levels of LDL-C and reduced incidence of ASCVD. In a study comparing the incidence of coronary heart disease over 15 years among individuals taking part in the Atherosclerosis Risk in Communities (ARIC) study, nonsense mutations in PCSK9 were associated with a 28% reduction in LDL-C and an 88% reduction in the rate of coronary events among African Americans [6]. Similarly, the R46L variant was associated with a 15% reduction in LDL-C and a 47% reduction in ASCVD risk in whites [6]. In a Japanese population, it has been reported that a PCSK9 R93C variant was associated with low LDL-C concentration [7]. In this regard, there has been the case of a 32-year-old African American woman who was a compound heterozygote for a PCSK9 loss of function mutation and although her LDL-C levels were markedly low at 14 mg/dL, she was not affected intellectually and graduated from university, was able to become pregnant and give birth and liver and renal function were normal [8]. Based on observations in the PCSK9-deficient mouse [9], loss of PCSK9 in mammals is not considered to influence viability or health.

Molecular biology research on PCSK9 has found that it promotes the degradation of LDL receptors by forming a complex with them, mainly in the liver. Localized on the cell surface, LDL receptors bind with LDL, and afterwards, the complex is transported to the endosomes via endocytosis and released the LDL under acidic conditions. LDL is degraded to amino acids and cholesterol while LDL receptors are transported to the cell surface, bind with LDL and taken into cells. This recycling of LDL receptors to the cell surface occurs approximately 150 times [10,11] (Fig. 1, left). PCSK9 is secreted by the endoplasmic reticulum in liver cells, binds with LDL receptors on the cell membrane, and is taken into cells. LDL receptors that PCSK9 has bound to are degraded in lysosomes without being recycled (Fig. 1, right).

Regarding gene mutations causing FH, the first to be discovered was that in the LDL receptor itself (FH1), next a mutation in apolipoprotein (apo) B (FH2), a ligand of LDL receptors, was discovered and the gain of function mutation in PCSK9 that promotes degradation of LDL receptors was the third one to be discovered and called FH3.

Relationship between statins and PCSK9 and difficulty of treating FH

Let us review the pharmacological action of HMG-CoA reductase inhibitors (statins) in order to better understand PCSK9 inhibitors. As shown in Fig. 2, cholesterol is mainly synthesized in the liver via the mevalonate pathway. The rate-limiting enzyme is HMG-CoA reductase and if there is a decrease in intracellular cholesterol in the liver, this is sensed by sterol regulatory-element binding protein 2 (SREBP2), a sensor molecule, and the expression of HMG-CoA reductase is increased to promote the intracellular synthesis of cholesterol. At the same time, intracellular cholesterol levels are elevated through increased expression of LDL receptors, which promotes intake of cholesterol from the blood. Statins are HMG-CoA reductase inhibitors and they decrease intracellular cholesterol by inhibiting cholesterol synthesis. Thus statins enhance SREBP2 activation and stimulate recovery of cholesterol from the blood via LDL receptors. As a result, the LDL-C level in the blood decreases and the intracellular cholesterol amount recovers.

<table>
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<tr>
<th>Phenotype</th>
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<td>LDL-C</td>
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FH, familial hypercholesterolemia; MTP, microsomal triglyceride transfer protein; LDLR, low-density lipoprotein receptor; ApoB, apolipoprotein B; PCSK9, proprotein convertase subtilisin/kexin type 9.
However, if statins are taken every day, intake of LDL from the blood will continue because LDL receptors are constantly expressed. Regarding intracellular cholesterol homeostasis, this is a big issue.

Interestingly, it has been found that SREBP2 also promotes the secretion of PCSK9. Thus, the body has an elaborate system to prevent LDL recovery from increasing more than necessary because the LDL receptors whose expression has been increased by statin are degraded by PCSK9. This partly explains the “6% rule of statin treatment”; meaning that LDL-C only decreases by about 6% even if the statin dose is doubled.

Fig. 1. LDL receptor pathway and LDL receptor degradation by PCSK9. LDL, low-density lipoprotein; PCSK9, proprotein convertase subtilisin/kexin type 9.

Fig. 2. Intracellular cholesterol regulating mechanism. HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; LDL, low-density lipoprotein; PCSK9, proprotein convertase subtilisin/kexin type 9; SREBP2, sterol regulatory-element binding protein 2.
Since LDL receptor degradation is enhanced by PCSK9, PCSK9 inhibition increases LDL-receptor recycling and LDL uptake. In view of homeostasis, co-administration of statin with PCSK9 inhibitor may disrupt a well-controlled intracellular cholesterol homeostasis due to high expression of LDL receptors caused by lack of degradation by PCSK9. This is also a big problem. Fortunately, another degradation system would work in order to maintain cholesterol level in the cell. Increased cholesterol in the hepatocytes not only down-regulates SREBP2 but also induces liver X receptor (LXR), leading to stimulate CYP7A1 and inducible degrader of the LDL receptor (IDOL) expressions. CYP7A1 is an enzyme that can convert cholesterol into bile acids. IDOL is an E3 ubiquitin ligase that triggers ubiquitination of the LDL receptor on its cytoplasmic domain, leading to targeting it for degradation (Fig. 3). Again, the body has an elaborate system to maintain intracellular cholesterol concentrations.

Fig. 4 shows the results for FH heterozygotes being treated as outpatients at our center before the launch of anti-PCSK9 monoclonal antibodies. Even for strong statin or statin combined with ezetimibe, the LDL-C management target for heterozygous FH patients of less than 100 mg/dL was achieved in just 16.2% of patients. It should be easy to understand that the effect of statin in increasing LDL receptor expression (LDL-C reducing effect) would be limited in FH patients with LDL receptor mutations or those that have PCSK9 gain of function mutations that promote LDL receptor degradation. Therefore, if statins and PCSK9 inhibitors are administered to heterozygous FH patients together, as the LDL receptors increased by statin would not be degraded by PCSK9, it is easy to see that there would be a greater increase in LDL-C levels.

New therapies targeting PCSK9

As stated above, PCSK9 degrades LDL receptor protein, so inhibiting PCSK9 will be an effective strategy. In many cases, the inhibitors being developed are small molecule compounds for oral administration but there has been great difficulty in their development. The reasons for this are considered to be stability in the blood of small molecule compounds and unstable efficacy, as well as difficulty in design due to the lack of a structure like an enzyme pocket for PCSK9 and LDL receptors to come together and interact. While it has been reported that berberine, a plant-derived substance, inhibits PCSK9 transcription [12], there are hardly any other studies on small molecule inhibitors.

Anti-PCSK9 monoclonal antibodies

Evolocumab and alirocumab are anti-PCSK9 antibodies that inhibit binding between PCSK9 and LDL receptors. They gained manufacturing and marketing approval in Japan in January and June 2016, respectively, and can now be prescribed. Adding an anti-PCSK9 antibody to standard therapy with statin alone or statin combined with ezetimibe reportedly further reduced serum LDL-C levels by around 60% and a retrospective analysis found that they halved cardiovascular event incidence as compared with placebo [13,14].

The GLAGOV study, whose results were presented in 2016, used intravascular ultrasound to evaluate the effect of adding evolocumab with respect to coronary artery plaque in coronary artery disease patients receiving statin therapy. While plaque volume increased by 0.05% in the placebo group, it decreased by 0.95% in the active drug group, demonstrating significant plaque regression efficacy for evolocumab. The mean LDL-C level in the active drug group was 36.6 mg/dL [15]. Also, the results of the FOURIER study, a large-scale outcome study in 27,564 ASCVD patients on statin therapy presented in 2017 showed that, with the addition of evolocumab, the mean LDL-cholesterol level dropped to 30 mg/dL. In addition, in a median follow-up period of 2.2 years, there was a decrease in the frequency of the composite primary endpoint of cardiovascular death, myocardial infarction, stroke, hospitalization due to unstable angina, and coronary artery revascularization (percutaneous coronary intervention, coronary artery bypass graft).

![Fig. 3. CYP7A1 activation by LXR and LDLR degradation by IDOL. IDOL, inducible degrader of the LDL receptor; LDLR, low-density lipoprotein receptor; LXR, liver X receptor.](image-url)
of 15%, compared with placebo. Regarding adverse reactions, while there were slightly more injection site reactions in the evolocumab group, no difference was observed between the groups regarding new-onset diabetes and cognitive impairment [16].

**Antisense oligonucleotides against PCSK9**

Antisense oligonucleotide agents that inhibit PCSK9 protein synthesis are being developed. They consist of nucleotides with complementary sequences to sense strands of RNA that code for proteins. Through the intraperitoneal administration of a PCSK9 antisense oligonucleotide in mice fed a high-fat diet, ISIS Pharmaceuticals (Carlsbad, CA, USA) was able to double LDL receptor expression in the liver and reduce LDL-C by 38% [17]. Also in our own development of a bridge nucleic acid-modified PCSK9 antisense agent with Obika et al., intraperitoneal administration in mice fed a high-fat diet achieved a dose-dependent reduction in PCSK9 in the liver and a decrease in LDL-C levels of 43% [18]. We are currently carrying out a preclinical study on this agent.

**siRNA against PCSK9**

Regarding ALN-PCS (inclisiran), a small interfering (or short interference) RNA (siRNA) for PCSK9, the results of a phase I clinical study in which it was administered to 32 healthy subjects [19] have been presented and following this, the results of the phase II Orion-1 study were presented at a meeting of the American Heart Association (AHA) in November 2016. In patients at high risk of cardiovascular disease due to high LDL-C levels despite optimal statin treatment, with a single subcutaneous injection of inclisiran 300 mg, mean LDL-C had decreased by 51% after 90 days and with a further injection of the same dose at 90 days after the first injection, LDL-C had decreased by 57% after 180 days. Inclisiran is considered to have good overall tolerability and among non-antibody based PCSK9 inhibitors, its development is proceeding most at present.

**Could PCSK9 antibody therapy be an alternative to apheresis?**

As the main mechanism of action of drug therapy for FH involves increasing LDL receptor expression in the liver, in FH homozygotes it is almost ineffective in decreasing LDL-C levels because they have no LDL receptor activity. Also, there are serious cases of FH among heterozygotes in whom progress of atherosclerosis cannot be controlled with drug therapy alone. In view of the necessity of overcoming such limitations of drug therapy, lipoprotein apheresis has been developed as a technique for removing LDL from plasma.

However, the problem with lipoprotein apheresis is that it imposes a great burden on the patient. The cost per treatment is high, it has to be performed at an interval of once every 1–2 weeks, and takes 3–5 hours each time. It has been said that FH in most homozygotes will lead to cardiovascular death by the age of 30 years if they do not undergo lipoprotein apheresis therapy but many drop out of therapy due to physical or financial problems or those related to participating in society. Also, while apheresis can delay the progression of atherosclerosis, it cannot stop it completely. Thus, in the current situation, when CAD develops in FH patients, they are at a disadvantage in society because of the high costs of in-hospital treatment and additional medication and being absent from work.

On the other hand, the very potent LDL-C lowering effect of anti-PCSK9 antibody therapies has enabled the frequency of apheresis to be reduced or to be discontinued [20]. This should be welcomed due to the reduced burden on the patient in physical terms as well as with regard to social disadvantage and the economic perspective. However, it has been reported that besides apo B-containing lipoproteins, apheresis removes inflammatory cytokines and adhesion molecules that are thought to cause atherosclerosis, and also improves the viscosity of the blood. In addition, we reported that PCSK9s were removed by lipoprotein apheresis in homozygous and heterozygous FH either by binding to apoB or by other mechanisms [21]. Therefore, future research...
should investigate whether these PCSK9 inhibitors have other actions besides LDL-C lowering that make them comparable to apheresis.

Physiological actions of PCSK9 other than that in the liver

Recently, it has been reported that PCSK9 is expressed not only in the liver but also in other tissues where it has various physiological actions. Besides the liver, PCSK9 is expressed in small intestine epithelial cells and is involved in lipid absorption. In this regard, it was found that serum triglycerides (TG) levels did not rise after fat loading in PCSK9-deficient mice [22]. Moreover, as the mechanism for the role of PCSK9 in this case, it has been assumed that it promotes the interaction of apo-A4-B48 and lipid via microsomal triglyceride transfer protein (MTP) during absorption of lipids (cholesterol and TG) by the small intestine [23].

PCSK9 is also expressed in vascular smooth muscle cells in atherosclerotic plaque. When it is secreted by vascular smooth muscle cells, PCSK9 degrades LDL receptors in macrophages and other peripheral cells [24]. It has also been reported that macrophages which have lost their LDL receptors are activated by inflammatory cytokines and cause inflammation in atherosclerotic plaque [25], and that PCSK9 decreases ATP-binding cassette transporter A1 (ABCA1) expression in macrophages and decreases cholesterol efflux mediated by high-density lipoprotein [26].

Furthermore, PCSK9 is expressed in the brain and kidneys where it has various physiological actions. Further research can be expected in this area.

Future prospects for PCSK9 inhibitors and issues to be addressed

It is now clear that PCSK9 is a key player in cholesterol metabolism and, at 10 years, the shortness of the period from its discovery to drug development reflects the high level of attention that it has received. As patients with a loss of function mutation in PCSK9 gene are healthy and have a low risk of developing ASCVD, expectations for the efficacy of PCSK9 inhibitors has been particularly high.

While relatively high cost can be given as a problem, the fact that PCSK9 inhibitors are able to reduce LDL-C dramatically even in large numbers of FH patients who could not achieve targets until now would lead a bright future. To ensure that these drugs are given to the patients who really need them, it is necessary to raise the diagnosis rate (an urgent issue in the treatment of FH in Japan) and family (cascade) screening has to be more actively conducted.

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