Review

PCSK9: A key factor modulating atherosclerosis

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Coronary artery disease (CAD) due to obstructive atherosclerosis is a leading cause of death and has been recognized as a worldwide health threat. Measures to decrease low-density lipoprotein cholesterol (LDL-C) levels are the cornerstone in the management of patients with atherosclerotic cardiovascular disease, particularly those with CAD, for over two decades. Proprotein convertase subtilisin/kexin type 9 (PCSK9), a newly recognized protein, plays a key role in cholesterol homeostasis by enhancing degradation of hepatic LDL receptor (LDLR). Interestingly, PCSK9 is also involved in the inflammatory process. Plasma PCSK9 and lipid or nonlipid cardiovascular risk factors are correlated, and the associations between PCSK9 with cardiovascular health and disease make this protein worthy of attention for the treatment of hyperlipidemia and atherosclerosis. Here, we provide an overview of the physiological role of PCSK9, which contributes to atherosclerosis, and provide data on PCSK9 as a novel pharmacological target. Clinical evidence shows that PCSK9 inhibition is as promising as statins as a target to treat CAD. The efficacy of these drugs may potentially enable effective CAD prophylaxis for more patients.

J Atheroscler Thromb, 2015; 22: 221-230.

Key words: Proprotein convertase subtilisin/kexin type 9, Hyperlipidemia, Atherosclerosis, Coronary artery disease

Introduction

Coronary artery disease (CAD) due to obstructive atherosclerosis is a life-threatening disease associated with severe morbidity and mortality[1, 2]. Dyslipidemia, particularly hypercholesterolemia, and maladaptive immune responses involving chronic inflammation of the arterial wall have been increasingly recognized as causal risk factors for the development of atherosclerosis[3]. Measures to decrease low-density lipoprotein cholesterol (LDL-C) levels are the cornerstone in the management of patients with atherosclerotic cardiovascular disease, particularly those with CAD, for over two decades. However, ≤ 80% of CAD is preventable according to the World Health Organization[1]. CAD risk can be decreased significantly by preventing or treating modifiable risk factors. Lipid-lowering treatments, particularly statin therapy, is one of the most important interventions, as indicated by the results of many landmark trials[4-6]. However, despite maximally tolerated statin therapy, some patients remain to have a considerable residual risk of cardiovascular events or failure to achieve the lipid goal[6]. The ongoing residual risk of clinical events has been highlighted by the need to develop novel agents targeting atherogenic and protective lipid factors to achieve more effective CAD prophylaxis.

Proprotein convertase subtilisin/kexin type 9 (PCSK9), a serine protease synthesized primarily in the liver, is a newly identified protein that shows promise as a target for treating dyslipidemia and atherosclerosis[7-9]. Since its discovery in 2003, PCSK9 has become a genetically validated target for autosomal-dominant hypercholesterolemia, a form of familial

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Received: August 19, 2014
Accepted for publication: October 8, 2014

hypercholesterolemia, in which neither the LDL receptor (LDLR) nor the apoprotein B (apoB) ligand binding domain show mutations[10]. Previous studies have demonstrated an association between changes in PCSK9 function and atherosclerosis. PCSK9 gain-of-function (GOF) mutations lead to higher LDL-C levels and an increased CAD risk, whereas PCSK9 loss-of-function (LOF) mutations are associated with lower LDL-C levels and decreased cardiovascular risk[9]. Several studies have been conducted on PCSK9 to understand its physiological and pathological roles in vascular biology. PCSK9 plays a key regulatory role in lipid metabolism by enhancing endosomal and lysosomal degradation of hepatic LDLR, resulting in increased plasma LDL-C levels[11]. In addition, evidence from experimental and clinical studies shows that PCSK9 may accelerate atherosclerosis and CAD by several mechanisms beyond degradation of hepatic LDLR[12, 13]. Importantly, pharmacological inhibition of PCSK9 efficiently decreases LDL-C levels and improves other lipid parameters; this effect persists in the presence of background statin therapy[14]. These findings suggest that this novel protein may be the next blockbuster target to combat hyperlipidemia and atherosclerosis.

In this article, we review current evidence for the role of PCSK9 in lipid metabolism and associated pathologies, with an emphasis on the harmful effects of PCSK9 in atherosclerosis, and provide data on PCSK9 inhibition as a novel pharmacological approach in the treatment of dyslipidemia and CAD.

Main Pathogenic Hypothesis of Atherosclerosis

Atherosclerosis causes CAD through slowly progressing formation of lesions and luminal narrowing of the arteries. The underlying pathology is complex and receives continued interest. It has been widely believed that atherosclerosis is characterized by an imbalance in lipid metabolism and a maladaptive inflammatory response of the arterial wall[15].

Atherosclerosis is initiated by endothelial dysfunction and structural alterations, which permit subendothelial accumulation of LDL[16]. Many potential injurious agents, including hemodynamic and biochemical factors, such as smoking, chronic hyperlipidemia, hypertension, hyperglycemia, infections, and immunological injury, lead to alterations in attachments between endothelial cells or those between endothelial cells and connective tissue[17]. This situation permits hemodynamic forces caused by the blood flow shear to increase and possibly cause focal endothelial desquamation, which is followed by the adhesion and aggregation of platelets and monocytes and chemokine release at the sites of injury. Infiltration by platelet-derived factors and plasma constituents, such as lipoproteins and/or metabolites, occur at these sites of injury and is accompanied by synthesis of new connective tissue matrix proteins as well as by lipid deposition and modification.

Oxidized or modified lipids trigger expression of adhesion molecules and secretion of chemokines by endothelial cells, which drive intimal inflammatory cell infiltration together with deposition of platelet-derived chemokines[15]. Early fatty-streak lesions consist of T cells and monocyte-derived macrophage-like foam cells loaded with lipid. The successive accumulation of apoptotic cells, debris, and cholesterol crystals forms a necrotic core. Fibroatheromatous plaques are covered by a fibrous cap composed of collagen and smooth muscle cells (SMCs), which are replaced by macrophages in the thinning inflamed caps that are prone to rupture. The shoulder regions are heavily infiltrated by T cells and mast cells, which produce enzymes and proinflammatory mediators, contributing to adventitial inflammation in advanced plaques.

The atherosclerosis field is so vast that attempts to comprehensively cover all aspects are futile. However, the knowledge that atherosclerosis is an inflammatory disease or a cholesterol-storage disease provides opportunities to achieve encouraging results against cardiovascular disease. To date, PCSK9 is the most promising and advanced approach to lower LDL-C levels and to prevent or treat atherosclerosis.

Experimental Evidence for PCSK9 in Atherogenesis

Experimental studies have identified a biological link between PCSK9 and the development of atherosclerosis. Inactivating PCSK9 protects wild-type and apoE-deficient mice models from atherosclerosis, whereas PCSK9 overexpression results in more severe atherosclerotic phenotypes[18]. PCSK9 is expressed in human atherosclerotic plaques and is secreted by vascular SMCs, possibly triggering lipid accumulation and modification[19]. Importantly, the harmful effects of PCSK9 on atherogenesis are mainly mediated via degradation of hepatic LDLR, but additional mechanisms, including inflammatory pathways, are of interest with regard to the impact PCSK9 on vascular biology (**Fig. 1**).

PCSK9 and Lipid Metabolism

PCSK9 is a secretory protein that regulates LDLR post-transcriptionally and has revolutionized

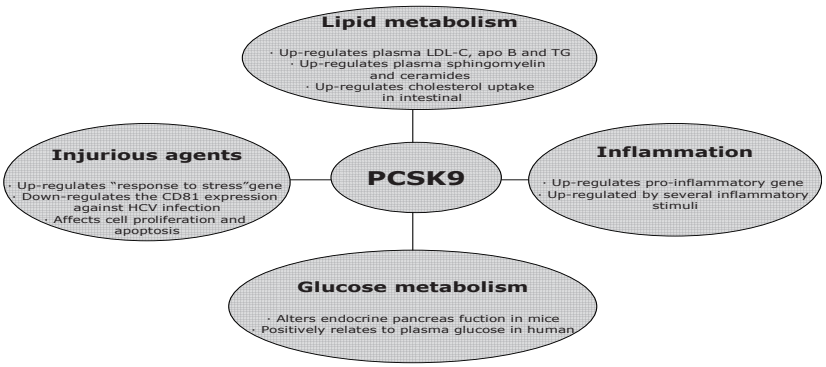

Fig. 1. Functions and properties of PCSK9. Important functions of PCSK9 include its roles in lipid metabolism, inflammation, glucose metabolism, and injury processes

Abbreviations: PCSK9, proprotein convertase subtilisin/kexin type 9; LDL-C, low-density lipoprotein cholesterol; apo B, apolipoprotein B; TG, triglycerides; HCV, hepatitis C virus.

our knowledge of the control of cholesterol homeostasis. PCSK9 is expressed predominantly in the liver, intestine, and kidneys, and its physiological relevance was revealed by identifying PCSK9 point mutations that cause hypercholesterolemia and hypocholesterolemia as a consequence of GOF and LOF alleles, respectively[20]. PCSK9 acts by enhancing degradation of hepatic LDLR, resulting in increased LDL-C levels. Interestingly, LDLR mutations that affect both LDL and PCSK9 clearance (e.g., receptor-negative mutations) aggravate hypercholesterolemia by their effects of accumulated PCSK9 on the normal LDLR[21]. Patients carrying such mutations would be particularly good targets for approaches of PCSK9 inhibition.

Adenoviral-mediated expression of human PCSK9 in mice results in an intermediate LDLR-knockout phenotype[22]. Although LDLR is not detected in the liver of such mice, circulating LDL-C only increases approximately fivefold when compared with the 15-fold increase in LDLR-knockout mice[23]. This finding suggests that LDLR in other tissues contribute to LDL-C clearance, and that LDLR in these extrahepatic tissues are not regulated by, or accessible to, circulating PCSK9. Annexin A2 levels are much lower in the liver than in other tissues. Therefore, it is tempting to speculate that Annexin A2 may act as a PCSK9 inhibitor, limiting the extrahepatic activity of the protein[24] In contrast, studies have shown increases in cholesterol levels in an LDLR-independent manner[21]. Up to 30% of circulating PCSK9 is associated with LDL, regardless of LDLR expression, probably by increasing both the hepatic and intestinal synthesis of apoB, the LDL particle structural protein[21]. Previous investigations involving cells, mice, and humans have suggested that PCSK9 may play a role in regulating apoB synthesis and secretion, and they have provided convincing evidence of a direct endogenous protein-protein interaction between PCSK9 and apoB[25]. Moreover, GOF forms of PCSK9, such as D374Y and S127R, have higher binding affinities for apoB than wild-type PCSK9[25-27]. The PCSK9/apoB interaction inhibits the intracellular degradation of apoB, regardless of LDLR, which results in the increased secretion of apoB and apoB-containing lipoproteins.

Furthermore, PCSK9 has effects on lipid metabolism in addition to its role with LDLR. It seems that other receptors, including known PCSK9 targets, such as very low-density lipoprotein receptor (VLDLR)[28], apoE receptor 2[28], lipoprotein receptor-related protein 1[29, 30], cluster of differentiation 36 (CD36)[30], or a yet unknown receptor, are also sensitive to PCSK9. Studies on PCSK9-knockout and PCSK9-transgenic mice reveal that PCSK9 downregulates the entry of triglycerides (TG) and free-fatty acids (FFAs) into visceral adipocytes possibly via adipose tissue VLDLR and CD36 degradation, resulting in decreased postprandial hypertriglyceridemia and enhanced chylomicron clearance[31]. Lipidomics analyses of PCSK9-knockout mice show a marked reduction in plasma sphingomyelin and ceramide levels, which are known risk factors for CAD[18]. A positive association is observed between plasma PCSK9 and TG in the general population, and inhibiting PCSK9 with a specific monoclonal antibody (mAb) is associated with a reduction in triglyceride levels[32]. In addition, treating

human intestinal epithelial cells with recombinant PCSK9 enhances cholesterol uptake, suggesting a role for PCSK9 in lipid absorption[30]. These effects of PCSK9 on lipid biomarkers suggest that PCSK9 is essential in lipid metabolism beyond LDL-C level regulation. The complex mechanism of action remains poorly understood but it has been shown to depend on intracellular trafficking.

PCSK9 and Inflammation

Gene expression studies in hepatoma cells have shown that PCSK9 overexpression regulates genes involved not only in cholesterol metabolism but also in apoptosis, inflammation, and proliferation[12]. Experimental data show that PCSK9 is markedly induced by diverse inflammatory stimuli, such as lipopolysaccharides, zymosan, and turpentine[33], resulting in a significant increase in LDL-C levels. Importantly, oxidized LDL (oxLDL), a major proinflammatory factor in the development of atherosclerosis, also increases PCSK9 expression by altering the secretion of inflammatory chemokines, such as interleukin-1α (IL-1α), IL-6, and tumor necrosis factor-α, in THP-1-derived macrophages in a dose-dependent manner[34]. Furthermore, siRNA-mediated knockdown of PCSK9 suppresses oxLDL-induced proinflammatory chemokine synthesis and secretion by inhibiting nuclear factor kappa B alpha degradation and nuclear factor kappa B translocation, which have an anti-inflammatory role in macrophages[34].

In our clinical study, we reportedthat plasma PCSK9 levels are associated with traditional markers of chronic low-grade inflammation, which are known CAD risk factors, such as circulating white blood cell count and platelet count[13, 35]. However, some studies have reported contrasting results. For example, the PCSK9 D374Y GOF mutation upregulates genes involved in sterol biosynthesis but downregulates expression of specific inflammatory pathways in HepG2 cells[36]. Further clarification of the functional role of PCSK9 is needed, and it may hasten its application as a unique atherosclerosis therapeutic target.

PCSK9 and Several Injurious Agents

PCSK9 affects several pathways, including cholesterol biosynthesis, stress response, and response to chemical stimuli[36]. Pathways in stress response are likely a direct effect of PCSK9, because multiple associated genes are upregulated by the GOF mutation of PCSK9[36]. Hepatitis C virus (HCV) infection is a major worldwide health issue because of chronic liver disease and extrahepatic manifestations including CAD[37]. Interestingly, PCSK9 could potentially protect against HCV in mice by downregulating the hepatic expression of CD81 and HCV entry receptors, resulting in a decreased HCV cellular infection rate, although this has not been shown in humans[38]. Moreover, PCSK9 deficiency results in delayed hepatocyte proliferation and enhanced apoptosis following partial hepatectomy, which can be rescued by feeding the mice a high-cholesterol diet[23]. These results indicate that patients with hepatic damage lacking PCSK9 could be at risk. However, a PCSK9 deficiency confers resistance to hepatosteatosis under normal conditions[23].

Notably, circulating PCSK9 appears to be a late biomarker of illness severity in patients with severe multiple trauma[39]. A case report showed toxic acute tubular injury in a healthy 56-year-old woman volunteer after the administration of a pharmacologically active dose of a locked nucleic acid antisense oligonucleotide against PCSK9[40]. Fortunately, 44 days after the last oligonucleotide dose was administered, the patient recovered fully, and kidney function was normal at every follow-up visit[40]. The underlying mechanisms are unknown, but an effect of PCSK9 in stress and tissue damage responses may be involved. Therefore, caution should be exercised when using PCSK9 inhibitors, because a careful evaluation of the risk-benefit ratio is needed, particularly in patients at risk of hepatorenal injury.

Clinical Evidence for PCSK9 in CAD

PCSK9 and Cardiometabolic Risk Factors

The function of PCSK9 as a secreted factor is physiologically significant. Thus, the measurement of circulating PCSK9 levels in humans is logical from a clinical perspective (**Table 1**). Identifying the correlations between plasma PCSK9 levels and lipid and nonlipid risk factors supports the hypothesis that PCSK9 may have a broader physiological role in the vascular system. Recent reports on blacks, nonhispanic whites, Canadians, and the Han Chinese population have revealed that several demographic and metabolic parameters, including age, glucose, obesity or body mass index (BMI), and blood pressure are positively correlated with PCSK9 level[41-43].

It is well recognized that diabetes is associated with accelerated atherosclerosis. PCSK9 has also been detected in the endocrine pancreas and more specifically in pancreatic δ cells[44]. PCSK9 alters endocrine pancreatic function in mice, and PCSK9 deficiency is associated with morphological abnormalities in β islets, resulting in decreased insulin secretion and

Table 1. Associations between circulating PCSK9 levels and CAD

Associations with	Number of subjects	Design	PCSK9 media, range	Author (reference)
Cardiovascular risk factors				
Age, $r=0.18$; BMI, $r=0.12$; SBP, $r=0.07$; DBP, $r=0.08$; LDL-C, $r=0.24$; TG, $r=0.25$; FBG, $r=0.17$; CRP, $r=0.11$.	3138 (1392 men and 1746 women)	Healthy subjects from the Dallas Heart Study	487 (33-2988) ng/ml	Lakoski *et al.* 2009. [41]
Age, $r=0.16$; BMI, $r=0.11$; SBP, $r=0.16$; DSP, $r=0.13$; LDL-C, $r=0.26$; TG, $r=0.25$; FBG, $r=0.12$.	2682 (1633 men and 1049 women)	Healthy subjects from Han Chinese	69.35 (12.85-222.5) ng/ml	Cui *et al.*, 2010. [43]
FBG, $\beta=5.5$; Insulin, $\beta=1.1$; HOMA-IR, $\beta=1.1$.	1739 (874 boys and 865 girls)	Healthy youth (9-16 years) from French Canadian	84.7 (17.6-211.7) μg/L	Baass *et al.*, 2009. [42]
Severity of CAD				
Carotid IMT, $r=0.242$.	127	Hypertensive patients from Korea	225.3 (69.35-600.7) ng/mL	Lee *et al*, 2013. [57]
Carotid IMT, $\beta=0.46$.	112	Subjects with negative FH mutation from Netherlands	177 (?) ng/ml	Huijgen *et al.*, 2012. [56]
Coronary Gensini score, $r=0.191$.	243	Patients with coronary atherosclerosis from China	218.77 (121.53-477.7) ng/ml	Li et al., 2014. [58]
CV outcome				
2 years follow-up (PCSK9 >622 ng/ml vs. <471 ng/ml: HR 1.55, 95% CI 1.11-2.16	504 (420 men and 84 women)	Stable CAD patients with statin controlled LDL from Germany	men 542 (?), women 607 (?) ng/ml	Werner *et al.*, 2014. [74]

r, correlation coefficient; β percent difference in the mean concentration of the dependent variable for a 1-unit increase (10% change) in the explanatory variable (PCSK9), and hazard ratio (HR) with 95% confidence interval (CI). Abbreviations: PCSK9, proprotein convertase subtilisin/kexin type 9; CAD, coronary artery disease; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, low density lipoprotein cholesterol; TG, triglyceride; FBG, fasting blood-glucose; CRP, C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance; IMT, intima-media thickness.

chronic hyperglycemia[45]. Circulating PCSK9 is positively correlated with fasting blood glucose levels and the homeostasis model assessment of insulin resistance, an index of insulin sensitivity, in several cohorts of nondiabetic subjects[41-43]. However, inhibiting PCSK9 with an mAb in clinical studies did not significantly alter glucose metabolism. We speculate that pharmacological interventions that severely decrease PCSK9 plasma levels would not produce such pancreatic dysfunction[46].

Circulating PCSK9 was positively associated with BMI in several cohorts[41-43]. PCSK9 null mice accumulate abdominal fat, suggesting a role for PCSK9 in adipogenesis[47]. This phenotype is independent of LDLR and mediated by VLDLR and CD36 targeted by PCSK9. Thus, PCSK9 is essential in fat metabolism because it maintains high circulating LDL-C levels via hepatic LDLR degradation and also downregulates triglyceride and FFA entry into visceral adipocytes.

Hypertension accelerates CAD progression. Recent studies show that PCSK9 induces degradation of epithelial Na(+) channels, which regulate blood pressure by modulating epithelial sodium reabsorption in the collecting ducts[48]. It is unclear whether this cellular observation is related to the regulation of blood pressure, but a large cohort study reported a correlation between blood pressure and PCSK9 level[43]. PCSK9 is expressed at a high level in the kidneys but its functional role in this organ remains to be determined.

PCSK9 and the Presence/Severity of CAD

Previous studies have demonstrated an association between functional changes in PCSK9 and CAD. Mutations in this gene are associated with hypocholesterolemia and hypercholesterolemia through LOF[49-51] and GOF[10-52]. Importantly, these mutations may be

involved in CAD risk[49, 51, 53, 54] and contribute to the severity of coronary artery atherosclerosis[55]. For example, the Atherosclerosis Risk in Communities study demonstrated that carriers of the PCSK9 LOF variants R46L and Y142X or C679X were respectively associated with a 15% (28%) reduction in mean LDL-C levels and a 47% (88%) reduction in cardiovascular risk during a 15-year follow-up[51]. The mechanism by which PCSK9 affects this phenotype could be related to its known effect on plasma LDL-C levels. It is also possible that PCSK9 itself may have a direct proatherogenic effect for at least two reasons. First, PCSK9 of vascular and hepatic origin may modulate the cellular composition of atherosclerotic lesions by directly affecting vascular cell function[19]. Second, the protective role of PCSK9 LOF mutations against CAD risk is greater than that expected by the effect of PCSK9 sequence variants on LDL-C levels[51].

Furthermore, two studies in patients with familial hypercholesterolemia and hypertension showed that circulating PCSK9 is inversely correlated with carotid intima–media thickness (IMT), a surrogate marker of preclinical atherosclerosis[56, 57]. Correlations between PCSK9 and IMT occur independently of the plasma lipid profile, suggesting that other factors beyond LDL-C levels contribute to the observed effect on atherosclerosis. In addition, results of a cross-sectional study currently underway at our center to determine whether plasma PCSK9 levels are associated with severity of coronary lesions in patients with CAD show a positive correlation between PCSK9 and the severity of coronary lesions independent of the lipid profile[58].

Finally, plasma PCSK9 levels are correlated with cardiovascular risk in the statin-naïve population[31]. However, on-treatment lipid levels, including apoB and apoAI levels, are no longer predictive, whereas the relative importance of nonlipid markers (e.g., age, gender, and smoking status) and non-HDL-C levels, which includes triglyceride levels, has increased[59-61]. Therefore, it is important to assess lipid metabolism mediators such as PCSK9 in patients receiving statins. A prospective cohort study showed that elevated PCSK9 plasma levels are associated with cardiovascular events in patients with stable CAD despite statin therapy and a well-controlled LDL-C level[61].

Clinical Implications of PCSK9 Targeting CAD

PCSK9 is a highly desirable candidate for inactivation to treat hypercholesterolemia and CAD, as indicated by the increasing experimental and clinical evidence mentioned above. The magnitude of the reduction in LDL-C levels with PCSK9 inhibitor treatment is much greater than that achieved with existing approved approaches.

Dubuc *et al*. reported that PCSK9 expression is upregulated in isolated hepatocytes treated with different statins[62], and a similar significant increase in plasma PCSK9 levels has been described in both mice[63] and humans[64, 65] treated with a statin or other lipid-lowering agent[66, 67]. These effects are mediated by activating sterol regulatory element-binding protein-2-related transcription, which regulates both LDLR and PCSK9[63, 68]. These findings support the rationale that combined therapy with a statin and a PCSK9 inhibitor may potentiate the cholesterol-lowering efficacy of statins. In addition, PCSK9 inhibition may successfully treat patients with hypercholesterolemia or an atherogenic lipid profile that fail to reach their individual LDL-C goal under classic lipid-lowering treatment[69]. These patients have the following characteristics: (i) those with a high risk of developing side effects to statins (myotoxicity and hepatotoxicity), particularly if high doses are necessary; (ii) those who are poor responders to statin therapy alone and do not reach the target LDL-C level despite treatment with the largest tolerated statin dose; and (iii) those with severe hypercholesterolemia, particularly patients who are heterozygous carriers of LDLR, APOB, or PCSK9 gene mutations. In addition, pharmacological inhibition of PCSK9 efficiently decreases LDL-C levels and improves other lipid parameters, such as ceramides and lipoprotein (a)[70].

Four factors have been identified as natural PCSK9 inhibitors, including LDL, Annexin A2, furin, and PC5/6A[14]. Moreover, several therapeutic approaches to inhibit PCSK9 have been proposed, including[14] inhibiting PCSK9 synthesis with gene-silencing agents, such as antisense oligonucleotides or small-interfering RNA, inhibiting PCSK9 binding to LDLR with a mAb, small peptide, or adnectin; or inhibiting PCSK9 autocatalytic processing with a small molecule inhibitor. Results of phase Ⅰ, Ⅱ, and Ⅲ studies of PCSK9 inhibitors have been published.

However, PCSK9 inhibition or downregulation may have adverse consequences under certain conditions[71]. For example, PCSK9 exhibits beneficial effects on liver regeneration after a hepatic insult; thus, caution should be exercised when using a PCSK9 inhibitor in patients suffering from hepatitis or liver cirrhosis[23, 38]. In addition, PCSK9 plays an important role sustaining the normal pancreatic islet function; thus, combined PCSK9 inhibitor/statin therapy may cause large increases in LDLR expression and increase lipid accumulation in pancreatic cells to

toxic levels[45]. Finally, orally administering a PCSK9 inhibitor is a very difficult approach[72, 73], considering the fact that approximately 50% patients discontinue orally statin therapy within the first year, let alone intravenous administration of a PCSK9 inhibitor.

Summary

In summary, multiple cardiovascular risk factors reinforce each other in their effects on the development of atherosclerosis. Good management of hyperlipidemia contributes to more effective prophylaxis from this life-threatening disease. PCSK9 has attracted immense scientific interest to target hyperlipidemia and CAD. The physiological role of PCSK9 in the LDL-C pathway has been extensively unraveled by both *in vitro* and *in vivo* approaches. The role of PCSK9 should be systematically investigated to help reveal the pathogenesis of atherosclerosis. Further investigations will certainly increase our knowledge and reassure clinicians in their day-to-day approach to patients with CAD.

Declaration of Interest

The authors have no conflict of interests.

Competition

None.

Contributions

Li S wrote the manuscript, and Li J-J reviewed/edited the manuscript. We thank Rui-Xia Xu, Yuan-Lin Guo, and Yan Zhang for their helpful discussions and critical review of the manuscript.

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