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PCSK9 and Lipid Metabolism

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Abstract

Purpose of review: The purpose of this review is to highlight the recent findings of one of the most promising therapeutic targets in low-density lipoprotein (LDL) cholesterol management, proprotein convertase subtilisin/kexin type 9 (PCSK9).

Recent findings: Endoplasmic reticulum (ER) cargo receptor, surfactant locus protein 4 (SURF4) interacts with PCSK9 and regulates its exit from ER and its secretion. Once secreted, PCSK9 binds to heparin sulfate proteoglycans on the hepatocyte surface and this binding is required for PCSK9–LDL receptor (LDLR) complex formation and LDLR degradation. Post-transcriptionally, recent work has shown that PCSK9 gets degraded in the lysosomes by activation of the glucagon receptor signaling, providing more data on the hormonal regulation of PCSK9. Finally, human studies with PCSK9 inhibitors offered more evidence on their benefits and safe use.

Summary: Recent work on the regulation of PCSK9 has enhanced our understanding of its biology, which may provide important information for future PCSK9-based therapies.

Keywords

proprotein convertase subtilisin kexin number 9; low-density lipoprotein cholesterol; glucagon; therapy

INTRODUCTION

Atherosclerotic cardiovascular disease (CVD) is the leading cause of death worldwide [1]. Large population studies demonstrated a positive correlation between elevated levels of low-density lipoprotein cholesterol (LDL-C) and atherosclerosis and lowering of LDL-C is the primary target of therapy [2]. Introduced in 1987, statin drugs were a major development in the treatment of hyperlipidemia worldwide [3]. By inhibiting Hmg-CoA-reductase, the rate-limiting enzyme in the mevalonate pathway, statins lower cholesterol synthesis. This decrease in cholesterol in liver activates sterol regulatory element-binding protein-2 (SREBP-2), which upregulates low-density lipoprotein receptor (LDLR) expression and thus decreases plasma LDL-C. Meta-analyses of randomized clinical trials of statin therapy revealed that each 1 mmol/L reduction in LDL-C results in ~20% reduction of global cardiovascular risk and decreases all-cause mortality by ~10% [4]. Despite their widespread use, approximately 10–20% of patients are noted to be “statin-intolerant” with myalgia and

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other musculoskeletal complaints noted as common side-effects warranting statin discontinuation [5]. Moreover, growing evidence suggests that statin use may increase the risk of new-onset type 2 diabetes in patients with established risk factors [6, 7]. These results prompted researchers to identify novel molecules that may be targeted safely and can be used as an alternative therapy for LDL-C management.

The discovery of the ninth member of the proprotein convertase family, proprotein convertase subtilisin/kexin type 9 (PCSK9) in 2003 led to a major change in the approach to CVD treatment [8]. PCSK9 is a liver-secreted protein that has the peculiar ability to interfere and block the natural recycling of LDLR, resulting in impaired LDL-C clearance from the plasma [9]. Mutations in the PCSK9 gene were first diagnosed in a French family and have been identified as the third genetic cause of familial hypercholesterolemia, making PCSK9 protein a great target for CVD treatment [10]. Humans carrying loss-of-function mutations in the *PCSK9* gene present with low LDL-C plasma levels and decreased CVD risk, while gain-of-function mutations of *PCSK9* are associated with high LDL-C levels and increased risk for CVD [10, 11].

Structure and function of PCSK9

Located on chromosome 1, *PCSK9* gene encodes a 692-amino acid secreted protein, composed of signal peptide (aa 1–30), prosegment (aa 31–152), catalytic (aa 153–454) and C-terminal cysteine-histidine-rich (aa 455–692) domains. PCSK9 protein is abundantly expressed in the liver [8]. Besides liver, PCSK9 is expressed in small intestine and kidney and recent work has suggested that PCSK9 is also expressed in human adipocytes and its mRNA levels in adipose tissue positively correlates with body mass index values [12].

After synthesis, PCSK9 proprotein is directed to the endoplasmic reticulum (ER) where the signal peptide (1–30) is removed. Different from all other members of proprotein convertase family, PCSK9 is unique in a way that the autocatalytically cleaved 14 kDa inhibitory prosegment noncovalently binds to the 60 kDa mature form of PCSK9 in the ER, which results in its trafficking to the Golgi and secretion as an inactive dimer complex. Given that PCSK9's self-cleavage in the ER is critical for its function, conditions that interfere with ER function largely affect PCSK9's fate. For example, ER stress-causing agents reduce PCSK9 secretion through retaining it in the ER [13]. Of note, recent work has shown that loss-of-function PCSK9 mutants accumulate in the ER without activating the ER stress response or apoptosis, raising the possibility that reducing PCSK9 secretion, via ER retention, may serve as a novel approach to lower plasma PCSK9 and LDL-C [14].

Coat protein complex II (COP II) is essential for the transport of many secreted proteins from the ER to the Golgi and recently surfactant locus protein 4 (SURF4) was identified as an ER cargo receptor that actively recruits PCSK9 into COPII vesicles and promotes the efficient ER exit and secretion of PCSK9 [15]. The absence of SURF4 results in accumulation of PCSK9 in the ER and impaired PCSK9 secretion, suggesting SURF4 as a novel therapeutic target for the treatment of hypercholesterolemia. Within the secretory pathway PCSK9 can interact with and degrade LDLR, which is prevented by PCSK9's binding to glucose-regulated protein 94 (GRP94) in the ER [16]. Upon secretion, the catalytic domain of PCSK9 interacts with the epidermal growth factor precursor homology

repeat-A (EGFA) region of LDLR at the cell surface. Asp residues in the ligand-binding repeat domain of LDLR also contribute to LDLR's binding to PCSK9 [17]. The PCSK9-LDLR complex enters the cells in clathrin-coated vesicles, which results in the degradation of LDLR in the lysosome [18]. It is noteworthy to mention that PCSK9 binding to LDLR has tissue specificity and liver is the most responsive tissue to this regulation, suggesting the requirement of a co-receptor specific for liver. A recent study has shown that heparin sulfate proteoglycans (HSPG), highly present on the surface of the hepatocytes, capture PCSK9 and this binding is required for PCSK9-LDLR complex formation and LDLR degradation [19]. Heparan-sulfate mimetics such as dextran sulfate, pentosane sulfate and suramin bind PCSK9 and inhibit PCSK9-mediated LDLR degradation. Heparin-like molecules can also interact with LDL-C and play an important role in LDL-C-induced inhibition of PCSK9 uptake into the cells [20].

Although the best-known function of PCSK9 is to target LDLR for lysosomal degradation in hepatocytes, which increases LDL-C and atherosclerotic CVD, PCSK9 may affect atherosclerosis development through its effects on endothelial and vascular smooth muscle cells [21, 22]. Moreover, platelet activation and blood clotting factor VIII (FVIII) levels were also shown to be regulated by PCSK9, suggesting that PCSK9 contributes to CVD etiology through regulating many different pathways [23]. Interestingly, several lines of evidence suggest that PCSK9 may have other pleiotropic properties like improving septic shock outcome [24]; however, these results were not supported by others and need further investigation [25].

PCSK9 regulation

The proximal promoter of PCSK9 gene contains a functional sterol regulatory element (SRE), which is required for sterol-responsive element binding protein-2 (SREBP-2) to induce *PCSK9* transcription [26]. SREBP-2 is a master regulator of cholesterol homeostasis in cells [27]. By inhibiting HMG-CoA reductase, statins induce SREBP-2 activity and paradoxically stimulate the expression of *PCSK9* mRNA, which attenuates their LDL-C-lowering effect [28]. *PCSK9* gene also contains a hepatocyte nuclear factor 1 α (HNF1 α) binding motif in the proximal region of its promoter (Figure 1A). HNF1 α levels are upregulated by statin treatment and downregulated by the plant-derived cholesterol-lowering compound berberine and activation of mechanistic target of rapamycin complex 1 (mTORC1) signaling pathway [26, 29]. Furthermore, NAD-dependent deacetylase SIRT6, through its interaction with FOXO3, modulates histone acetylation of *Pcsk9*, thereby suppressing its transcription [30]. Recently, E2F1, a transcription factor controlling cell cycle, has been shown to bind the *Pcsk9* promoter and regulate *Pcsk9* mRNA levels. E2F1 deficient mice have lower PCSK9 in liver, which results in lower plasma LDL-C levels supporting the hypothesis that E2F1 might be a promising therapeutic target for plasma LDL-C management [31]. Besides these transcription factors, recent work has shown that thyroid-stimulating hormone (TSH) increases *PCSK9* mRNA expression in HepG2 cell line (Figure 1A). Moreover, circulating TSH levels in subclinical hypothyroid patients correlate with plasma LDL-C and PCSK9, suggesting a role for TSH in cholesterol metabolism [32]. Finally, the major metabolite of berberine, berberrubine, has been proposed to exert potent inhibitory effect on PCSK9 through a pathway involving extracellular signal regulated

kinase (ERK), which results in higher LDLR levels in cellular models, awaiting in vivo testing [33]. Post-transcriptionally, miR-191, miR-222 and miR-224 has been recently shown to directly interact with the 3'-UTR of *PCSK9* mRNA and regulate PCSK9 expression (Figure 1B) [34]. Post-translationally, PCSK9 gets regulated via Furin and PC5/6. Furin-cleaved shorter fragment of PCSK9 has less LDLR-binding capacity and is unable to degrade it [35]. In addition to this regulation, recent work has suggested that PCSK9 gets phosphorylated on its Ser 688 residue, which is decreased by chronic statin treatment; however, the significance of this finding remains to be investigated [36].

Besides these, PCSK9 has been shown to be hormonally regulated by insulin and estrogens, mostly at the mRNA level [37-39]. Interestingly, PCSK9 levels are reduced by fasting; however, in-depth mechanistic information is lacking [40]. Glucagon is the major fasting hormone that contributes to energy metabolism through regulating the hepatic glucose production during hypoglycemia. Glucagon also participates in the regulation of cholesterol metabolism as glucagon administration lowers plasma LDL-C and glucagon receptor antagonists developed for type 2 diabetes management increase LDL-C [41-45]. Consistent with this, recent work describing an inactivating glucagon receptor mutation with nearly complete inhibition of glucagon action in a pediatric case reported elevated LDL-C levels [46]. However, the underlying mechanism(s) hasn't been fully uncovered. In this context, our recent findings shed some light on this mechanism and showed that glucagon regulates plasma LDL-C through PCSK9 [47]. Silencing of hepatic glucagon receptor or inhibition of glucagon action resulted in increased plasma PCSK9 and LDL-C; whereas, glucagon treatment of WT mice had the opposite effects. Mechanistically, we showed that glucagon, acting through its downstream mediators EPAC2 (exchange protein activated by cAMP 2) and RAP1 (Ras-related protein 1), enhanced lysosomal degradation of PCSK9 without an effect on *Pcsk9* mRNA levels (Figure 1C). Our results provided novel insights into the molecular interplay between hepatic glucagon signaling and LDL-C metabolism and described a new posttranscriptional mechanism of PCSK9 regulation. It is important to note that previous work reported the involvement of lysosomal degradation in PCSK9 regulation and glucagon has been shown to stimulate lysosomal degradation pathways [48-50]. The mechanism(s) linking glucagon receptor signaling to lysosome-mediated degradation of PCSK9 remains to be elucidated.

PCSK9 inhibition as a therapy for lowering LDL-C

The great interest in PCSK9 research over the past 15 years is mainly due to its rapid and efficient translation from basic science to clinical practice. Fully human monoclonal antibodies against PCSK9 (alirocumab and evolocumab) that disrupt the binding of extracellular PCSK9 to LDLR are now approved by US Food and Drug Administration for use in patients with familial hypercholesterolemia and in patients with clinical atherosclerotic CVD who require additional lowering of LDL-C levels [51]. Clinical trials have demonstrated that treatment with these anti-PCSK9 antibodies results in ~60% lowering of LDL-C and protects against cardiac ischemic events (including myocardial infarction) and induces atheroma regression when combined with statins [52, 53]. Moreover, in patients with homozygous and heterozygous familial hypercholesterolemia who respond poorly to high-dose statins, treatment with PCSK9 antibodies further lowered LDL-C

suggesting the idea that PCSK9 inhibition may improve cardiovascular health and life expectancy of these patients who have very high LDL-C levels [54, 55].

Both alirocumab and evolocumab are well-tolerated and current data indicate that PCSK9 inhibitors do not result in increased risk for adverse events such as an increase in new-onset type 2 diabetes, liver toxicity or impairment in cognitive function even in patients whose LDL-C levels were lowered less than 20 mg/dl [56, 57]. These data suggest that extremely low plasma LDL-C with PCSK9 inhibition is safe. Interestingly, anti-PCSK9 antibodies led to significant dose-related reductions in plasma levels of Lipoprotein (a) [Lp(a)], which contains apolipoprotein B-100 (apoB) covalently bound to a plasminogen-like glycoprotein, apolipoprotein (a) [58]. Lp (a) is encoded by the *LPA* gene and is now recognized as an independent cause of atherosclerotic cardiovascular disease [59]. Although atorvastatin was shown to paradoxically increase plasma Lp(a), PCSK9 neutralization led to ~30% lowering of circulating Lp(a), which further supports a beneficial role for PCSK9 inhibition in CVD prevention [60]. Despite these beneficial effects of anti-PCSK9 antibodies, the need for monthly subcutaneous injections and their high-cost have limited their wide-spread use [61]. In this context, recent work using long-acting small interfering RNA directed against hepatic PCSK9 have shown significant reductions in LDL-C comparable to anti-PCSK9 antibody treatment [62]. Moreover, this RNA interference approach is more durable and has the potential to improve adherence without serious side effects [63]. However, long-term follow-up on the effectiveness and safety of this approach is needed. Currently, alternative approaches like small molecule inhibitors of PCSK9, PCSK9 vaccination or gene editing are under development to target PCSK9, which may bring alternative modalities to lower LDL-C if translated into humans [64-67]. Recent identification of a targetable groove adjacent to the EGF(A)-binding site of PCSK9 provided the proof of concept that future small molecule inhibitors of PCSK9 is feasible [64].

CONCLUSION

PCSK9 is an important player in cholesterol homeostasis. By binding to hepatic LDLR and promoting its degradation, PCSK9 reduces LDL-C uptake, leading to an increase in plasma LDL-C concentrations. The exciting new findings on its biology and function has broaden our knowledge; however, more mechanistic data is needed, which could potentially be harnessed for therapeutic intervention and could guide the use of anti-PCSK9 therapies in different patient populations. Recent data on the efficacy and tolerability of PCSK9 inhibitors is promising and accumulating evidence suggest that PCSK9 inhibition has many beneficial effects besides LDL-C management like lowering Lp(a) levels. Long-term safety data on anti-PCSK9 therapies in clinical practice will provide a therapeutic option for managing dyslipidemia.

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REFERENCES AND RECOMMENDED READING

- [1]. Fan J, Song Y, Chen Y et al. Combined effect of obesity and cardio-metabolic abnormality on the risk of cardiovascular disease: a meta-analysis of prospective cohort studies. *Int J Cardiol* 2013; 168:4761–4768. [PubMed: 23972953]
- [2]. Ference BA, Ginsberg HN, Graham I et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur Heart J* 2017; 38:2459–2472. [PubMed: 28444290]
- [3]. Goldstein JL, Brown MS. A century of cholesterol and coronaries: from plaques to genes to statins. *Cell* 2015; 161:161–172. [PubMed: 25815993]
- [4]. Cholesterol Treatment Trialists C, Baigent C, Blackwell L et al. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. *Lancet* 2010; 376:1670–1681. [PubMed: 21067804]
- [5]. Ahmad Z. Statin intolerance. *Am J Cardiol* 2014; 113:1765–1771. [PubMed: 24792743]
- [6]. Ference BA, Robinson JG, Brook RD et al. Variation in PCSK9 and HMGCR and Risk of Cardiovascular Disease and Diabetes. *N Engl J Med* 2016; 375:2144–2153. [PubMed: 27959767]
- [7]. Swerdlow DI, Preiss D, Kuchenbaecker KB et al. HMG-coenzyme A reductase inhibition, type 2 diabetes, and bodyweight: evidence from genetic analysis and randomised trials. *Lancet* 2015; 385:351–361. [PubMed: 25262344]
- [8]. Seidah NG, Benjannet S, Wickham L et al. The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): liver regeneration and neuronal differentiation. *Proc Natl Acad Sci U S A* 2003; 100:928–933. [PubMed: 12552133]
- [9]. Seidah NG. The PCSK9 revolution and the potential of PCSK9-based therapies to reduce LDL-cholesterol. *Glob Cardiol Sci Pract* 2017; 2017:e201702. [PubMed: 28971102] ••This article gives an extensive review of the history of work done on PCSK9 biology and recent work on PCSK9 inhibition as therapeutics.
- [10]. Abifadel M, Varret M, Rabes JP et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. *Nat Genet* 2003; 34:154–156. [PubMed: 12730697]
- [11]. Kent ST, Rosenson RS, Avery CL et al. PCSK9 Loss-of-Function Variants, Low-Density Lipoprotein Cholesterol, and Risk of Coronary Heart Disease and Stroke: Data From 9 Studies of Blacks and Whites. *Circ Cardiovasc Genet* 2017; 10:e001632. [PubMed: 28768753]
- [12]. Bordicchia M, Spannella F, Ferretti G et al. PCSK9 is Expressed in Human Visceral Adipose Tissue and Regulated by Insulin and Cardiac Natriuretic Peptides. *Int J Mol Sci* 2019; 20. •This study suggests that PCSK9 is expressed in human adipocytes and its expression correlates with the body mass index.
- [13]. Lebeau P, Al-Hashimi A, Sood S et al. Endoplasmic Reticulum Stress and Ca²⁺ Depletion Differentially Modulate the Sterol Regulatory Protein PCSK9 to Control Lipid Metabolism. *J Biol Chem* 2017; 292:1510–1523. [PubMed: 27909053] •This study has shown that activation of endoplasmic reticulum stress inhibits PCSK9 secretion, which results in reduced PCSK9-mediated LDLR degradation.
- [14]. Lebeau P, Platko K, Al-Hashimi AA et al. Loss-of-function PCSK9 mutants evade the unfolded protein response sensor GRP78 and fail to induce endoplasmic reticulum stress when retained. *J Biol Chem* 2018; 293:7329–7343. [PubMed: 29593095] •This work revealed that loss-of-function PCSK9 mutants are retained in the endoplasmic reticulum (ER) without activating the ER stress response.
- [15]. Emmer BT, Hesketh GG, Kotnik E et al. The cargo receptor SURF4 promotes the efficient cellular secretion of PCSK9. *Elife* 2018; 7. •This study identified surfactant locus protein 4 (SURF4) as an endoplasmic reticulum (ER) cargo receptor that actively recruits PCSK9 into COPII vesicles and promotes the efficient ER exit and secretion of PCSK9.
- [16]. Poirier S, Mamarbachi M, Chen WT et al. GRP94 Regulates Circulating Cholesterol Levels through Blockade of PCSK9-Induced LDLR Degradation. *Cell Rep* 2015; 13:2064–2071. [PubMed: 26628375]

- [17]. Deng SJ, Alabi A, Gu HM et al. Identification of amino acid residues in the ligand binding repeats of LDLR important for PCSK9 binding. *J Lipid Res* 2019. •This article identified Asp amino acid residues in the ligand-binding repeat domain of LDLR, which mediate LDLR's binding to PCSK9.
- [18]. Horton JD, Cohen JC, Hobbs HH. Molecular biology of PCSK9: its role in LDL metabolism. *Trends Biochem Sci* 2007; 32:71–77. [PubMed: 17215125]
- [19]. Gustafsen C, Olsen D, Vilstrup J et al. Heparan sulfate proteoglycans present PCSK9 to the LDL receptor. *Nat Commun* 2017; 8:503. [PubMed: 28894089] •This work revealed that heparin sulfate proteoglycans (HSPG) capture PCSK9 in liver and this binding is required for PCSK9-LDLR complex formation and LDLR degradation.
- [20]. Galvan AM, Chorba JS. Cell-associated heparin-like molecules modulate the ability of LDL to regulate PCSK9 uptake. *J Lipid Res* 2019; 60:71–84. [PubMed: 30463987]
- [21]. Sun H, Krauss RM, Chang JT, Teng BB. PCSK9 deficiency reduces atherosclerosis, apolipoprotein B secretion, and endothelial dysfunction. *J Lipid Res* 2018; 59:207–223. [PubMed: 29180444]
- [22]. Karagiannis AD, Liu M, Toth PP et al. Pleiotropic Anti-atherosclerotic Effects of PCSK9 Inhibitors From Molecular Biology to Clinical Translation. *Curr Atheroscler Rep* 2018; 20:20. [PubMed: 29525934]
- [23]. Paciullo F, Momi S, Gesele P. PCSK9 in Haemostasis and Thrombosis: Possible Pleiotropic Effects of PCSK9 Inhibitors in Cardiovascular Prevention. *Thromb Haemost* 2019.
- [24]. Walley KR, Thain KR, Russell JA et al. PCSK9 is a critical regulator of the innate immune response and septic shock outcome. *Sci Transl Med* 2014; 6:258ra143.
- [25]. Berger JM, Loza Valdes A, Gromada J et al. Inhibition of PCSK9 does not improve lipopolysaccharide-induced mortality in mice. *J Lipid Res* 2017; 58:1661–1669. [PubMed: 28600283] •This work presents data suggesting that PCSK9 deficiency does not protect mice from lipopolysaccharide-induced death and shows evidence that there is no correlation between plasma inflammation markers with PCSK9 levels in a human cohort.
- [26]. Li H, Dong B, Park SW et al. Hepatocyte nuclear factor 1alpha plays a critical role in PCSK9 gene transcription and regulation by the natural hypocholesterolemic compound berberine. *J Biol Chem* 2009; 284:28885–28895. [PubMed: 19687008]
- [27]. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* 2002; 109:1125–1131. [PubMed: 11994399]
- [28]. Dubuc G, Chamberland A, Wassef H et al. Statins upregulate PCSK9, the gene encoding the proprotein convertase neural apoptosis-regulated convertase-1 implicated in familial hypercholesterolemia. *Arterioscler Thromb Vasc Biol* 2004; 24:1454–1459. [PubMed: 15178557]
- [29]. Ai D, Chen C, Han S et al. Regulation of hepatic LDL receptors by mTORC1 and PCSK9 in mice. *J Clin Invest* 2012; 122:1262–1270. [PubMed: 22426206]
- [30]. Tao R, Xiong X, DePinho RA et al. FoxO3 transcription factor and Sirt6 deacetylase regulate low density lipoprotein (LDL)-cholesterol homeostasis via control of the proprotein convertase subtilisin/kexin type 9 (Pcsk9) gene expression. *J Biol Chem* 2013; 288:29252–29259. [PubMed: 23974119]
- [31]. Lai Q, Giralt A, Le May C et al. E2F1 inhibits circulating cholesterol clearance by regulating Pcsk9 expression in the liver. *JCI Insight* 2017; 2. •This article shows that E2F1 binds to Pcsk9 promoter and regulates Pcsk9 mRNA levels.
- [32]. Gong Y, Ma Y, Ye Z et al. Thyroid stimulating hormone exhibits the impact on LDLR/LDL-c via up-regulating hepatic PCSK9 expression. *Metabolism* 2017; 76:32–41. [PubMed: 28987238]
- [33]. Cao S, Xu P, Yan J et al. Berberubine and its analog, hydroxypropyl-berberubine, regulate LDLR and PCSK9 expression via the ERK signal pathway to exert cholesterol-lowering effects in human hepatoma HepG2 cells. *J Cell Biochem* 2018.
- [34]. Naeli P, Mirzadeh Azad F, Malakootian M et al. Post-transcriptional Regulation of PCSK9 by miR-191, miR-222, and miR-224. *Front Genet* 2017; 8:189. [PubMed: 29230236] •This study reveals that miR-191, miR-222, and miR-224 directly interact with the 3'-UTR of Pcsk9 mRNA and regulate PCSK9 expression.

- [35]. Benjannet S, Rhainds D, Hamelin J et al. The proprotein convertase (PC) PCSK9 is inactivated by furin and/or PC5/6A: functional consequences of natural mutations and post-translational modifications. *J Biol Chem* 2006; 281:30561–30572. [PubMed: 16912035]
- [36]. Gauthier MS, Awan Z, Bouchard A et al. Posttranslational modification of proprotein convertase subtilisin/kexin type 9 is differentially regulated in response to distinct cardiometabolic treatments as revealed by targeted proteomics. *J Clin Lipidol* 2018; 12:1027–1038. [PubMed: 29699916]
- [37]. Persson L, Henriksson P, Westerlund E et al. Endogenous estrogens lower plasma PCSK9 and LDL cholesterol but not Lp(a) or bile acid synthesis in women. *Arterioscler Thromb Vasc Biol* 2012; 32:810–814. [PubMed: 22207727]
- [38]. Costet P, Cariou B, Lambert G et al. Hepatic PCSK9 expression is regulated by nutritional status via insulin and sterol regulatory element-binding protein 1c. *J Biol Chem* 2006; 281:6211–6218. [PubMed: 16407292]
- [39]. Miao J, Manthena PV, Haas ME et al. Role of Insulin in the Regulation of Proprotein Convertase Subtilisin/Kexin Type 9. *Arterioscler Thromb Vasc Biol* 2015; 35:1589–1596. [PubMed: 26023080]
- [40]. Browning JD, Horton JD. Fasting reduces plasma proprotein convertase, subtilisin/kexin type 9 and cholesterol biosynthesis in humans. *J Lipid Res* 2010; 51:3359–3363. [PubMed: 20716520]
- [41]. Guettet C, Mathe D, Navarro N, Lecuyer B. Effects of chronic glucagon administration on rat lipoprotein composition. *Biochim Biophys Acta* 1989; 1005:233–238. [PubMed: 2804052]
- [42]. Amatuzio DS, Grande F, Wada S. Effect of glucagon on the serum lipids in essential hyperlipemia and in hypercholesterolemia. *Metabolism* 1962; 11:1240–1249. [PubMed: 14012500]
- [43]. Kazierad DJ, Bergman A, Tan B et al. Effects of multiple ascending doses of the glucagon receptor antagonist PF-06291874 in patients with type 2 diabetes mellitus. *Diabetes Obes Metab* 2016; 18:795–802. [PubMed: 27059951]
- [44]. Engel SS, Reitman ML, Xu L et al. Glycemic and Lipid Effects of the Short-Acting Glucagon Receptor Antagonist MK-3577 in Patients With Type 2 Diabetes. *Diabetes* 2012; 61:A266–A266.
- [45]. Guzman CB, Zhang XM, Liu R et al. Treatment with LY2409021, a glucagon receptor antagonist, increases liver fat in patients with type 2 diabetes. *Diabetes Obes Metab* 2017; 19:1521–1528. [PubMed: 28371155]
- [46]. Li H, Zhao L, Singh R et al. The first pediatric case of glucagon receptor defect due to biallelic mutations in GCGR is identified by newborn screening of elevated arginine. *Mol Genet Metab Rep* 2018; 17:46–52. [PubMed: 30294546]
- [47]. Spolitu S, Okamoto H, Dai W et al. Hepatic Glucagon Signaling Regulates PCSK9 and Low-Density Lipoprotein Cholesterol. *Circ Res* 2019; 124:38–51. [PubMed: 30582457] •This study suggests that glucagon receptor signaling, through its downstream effectors, Epac2 (exchange protein activated by cAMP 2) and Rap1 (Ras-related protein 1), enhances lysosomal degradation of PCSK9 and contributes to plasma LDL-C regulation.
- [48]. Wang Y, Huang Y, Hobbs HH, Cohen JC. Molecular characterization of proprotein convertase subtilisin/kexin type 9-mediated degradation of the LDLR. *J Lipid Res* 2012; 53:1932–1943. [PubMed: 22764087]
- [49]. Deter RL, Baudhuin P, De Duve C. Participation of lysosomes in cellular autophagy induced in rat liver by glucagon. *J Cell Biol* 1967; 35:C11–16. [PubMed: 6055998]
- [50]. Hopgood MF, Clark MG, Ballard FJ. Protein degradation in hepatocyte monolayers. Effects of glucagon, adenosine 3':5'-cyclic monophosphate and insulin. *Biochem J* 1980; 186:71–79. [PubMed: 6245643]
- [51]. Shapiro MD, Tavori H, Fazio S. PCSK9: From Basic Science Discoveries to Clinical Trials. *Circ Res* 2018; 122:1420–1438. [PubMed: 29748367] ••This review covers the latest therapies aimed at inhibiting PCSK9 in the clinical setting.
- [52]. Sabatine MS, Giugliano RP, Keech AC et al. Evolocumab and Clinical Outcomes in Patients with Cardiovascular Disease. *N Engl J Med* 2017; 376:1713–1722. [PubMed: 28304224]
- [53]. Schwartz GG, Steg PG, Szarek M et al. Alirocumab and Cardiovascular Outcomes after Acute Coronary Syndrome. *N Engl J Med* 2018; 379:2097–2107. [PubMed: 30403574]

- [54]. Hartgers ML, Defesche JC, Langslet G et al. Alirocumab efficacy in patients with double heterozygous, compound heterozygous, or homozygous familial hypercholesterolemia. *J Clin Lipidol* 2018; 12:390–396 e398. [PubMed: 29396260]
- [55]. Raal FJ, Honarpour N, Blom DJ et al. Inhibition of PCSK9 with evolocumab in homozygous familial hypercholesterolaemia (TESLA Part B): a randomised, double-blind, placebo-controlled trial. *Lancet* 2015; 385:341–350. [PubMed: 25282520]
- [56]. Giugliano RP, Pedersen TR, Park JG et al. Clinical efficacy and safety of achieving very low LDL-cholesterol concentrations with the PCSK9 inhibitor evolocumab: a prespecified secondary analysis of the FOURIER trial. *Lancet* 2017; 390:1962–1971. [PubMed: 28859947]
- [57]. Giugliano RP, Mach F, Zavitz K et al. Cognitive Function in a Randomized Trial of Evolocumab. *N Engl J Med* 2017; 377:633–643. [PubMed: 28813214] •This randomized trial in humans receiving either evolocumab or placebo in addition to statin therapy shows no significant changes in cognitive function over 19 months.
- [58]. Gaudet D, Kereiakes DJ, McKenney JM et al. Effect of alirocumab, a monoclonal proprotein convertase subtilisin/kexin 9 antibody, on lipoprotein(a) concentrations (a pooled analysis of 150 mg every two weeks dosing from phase 2 trials). *Am J Cardiol* 2014; 114:711–715. [PubMed: 25060413]
- [59]. O'Donoghue ML, Fazio S, Giugliano RP et al. Lipoprotein(a), PCSK9 Inhibition and Cardiovascular Risk: Insights from the FOURIER Trial. *Circulation* 2018. •This work reports that in Evolocumab treatment significantly lowers plasma Lp(a).
- [60]. Arsenault BJ, Petrides F, Tabet F et al. Effect of atorvastatin, cholesterol ester transfer protein inhibition, and diabetes mellitus on circulating proprotein subtilisin kexin type 9 and lipoprotein(a) levels in patients at high cardiovascular risk. *J Clin Lipidol* 2018; 12:130–136. [PubMed: 29103916]
- [61]. Fonarow GC, Keech AC, Pedersen TR et al. Cost-effectiveness of Evolocumab Therapy for Reducing Cardiovascular Events in Patients With Atherosclerotic Cardiovascular Disease. *JAMA Cardiol* 2017; 2:1069–1078. [PubMed: 28832867]
- [62]. Fitzgerald K, White S, Borodovsky A et al. A Highly Durable RNAi Therapeutic Inhibitor of PCSK9. *N Engl J Med* 2017; 376:41–51. [PubMed: 27959715] ••This is the first study reporting that RNA interference against PCSK9 safely lowers plasma PCSK9 and LDL-C for at least 6 months.
- [63]. Ray KK, Landmesser U, Leiter LA et al. Inclisiran in Patients at High Cardiovascular Risk with Elevated LDL Cholesterol. *N Engl J Med* 2017; 376:1430–1440. [PubMed: 28306389]
- [64]. Zhang Y, Ultsch M, Skelton NJ et al. Discovery of a cryptic peptide-binding site on PCSK9 and design of antagonists. *Nat Struct Mol Biol* 2017; 24:848–856. [PubMed: 28825733]
- [65]. Kawakami R, Nozato Y, Nakagami H et al. Development of vaccine for dyslipidemia targeted to a proprotein convertase subtilisin/kexin type 9 (PCSK9) epitope in mice. *PLoS One* 2018; 13:e0191895. [PubMed: 29438441]
- [66]. Seidah NG, Prat A, Pirillo A et al. Novel strategies to target proprotein convertase subtilisin kexin 9: beyond monoclonal antibodies. *Cardiovasc Res* 2019; 115:510–518. [PubMed: 30629143]
- [67]. Chadwick AC, Wang X, Musunuru K. In Vivo Base Editing of PCSK9 (Proprotein Convertase Subtilisin/Kexin Type 9) as a Therapeutic Alternative to Genome Editing. *Arterioscler Thromb Vasc Biol* 2017; 37:1741–1747. [PubMed: 28751571]

Key points:

- LDL-C management is protective against atherosclerotic CVD.
- PCSK9 is a novel therapeutic target that degrades LDLR and contributes to LDL-C homeostasis.
- Inhibition of PCSK9 not only lowers LDL-C but also reduces atherogenic Lp(a) in the plasma.
- Current data suggest that PCSK9 inhibition via monoclonal antibody injection and RNA interference is safe.

Figure 1. Molecular mechanisms of PCSK9 regulation.

(A) Statins, berberine, insulin, mTORC1, SIRT6, thyroid stimulating hormone (TSH), E2F1 and berberrubine regulate *PCSK9* mRNA expression through various mechanisms. (B) miR-191, miR-222 and miR-224 directly interact with the 3'-UTR of *PCSK9* mRNA and contribute to PCSK9 regulation. (C) Glucagon receptor signaling, through its downstream effectors, Epac2 (exchange protein activated by cAMP 2) and Rap1 (Ras-related protein 1), enhances lysosomal degradation of PCSK9.