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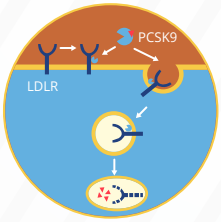
Proprotein Convertase Subtilisin/Kexin Type 9 in Cholesterol Homeostasis, Inflammation, and Thrombosis Associated with Cardiovascular Diseases

An overview of molecular functions and therapeutic strategies

Proprotein convertase subtilisin/kexin type 9 (PCSK9)[1]



- A serine endoprotease enzyme
- Recognises paired or multiple basic clusters or hydrophobic motifs
- Catalyses proteolytic activation, modification, and degradation of secreted proteins
- Primarily produced in the liver and small intestine

Protein domains and processing[1]

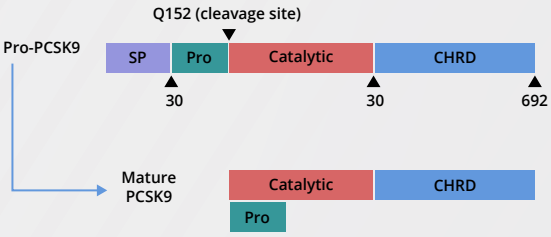
692 amino acid (aa) residue, 74.3 kDa zymogen (precursor enzyme) with three distinct domains

- Pro-domain (aa 31–152)
- Catalytic domain (aa 153–421) with a hinge (aa 422–452)
- Cysteine/histidine rich C-terminal domain (CHRD; aa 453–692) with three modules:

M1 (aa 453–529)

M2 (aa 530–603)

M3 (aa 604–692)



The pro-domain remains associated, non-covalently, with the catalytic domain, inhibiting its catalytic activity[1]

Functions

Cholesterol homeostasis
PCSK9-mediated degradation of low-density lipoprotein receptor (LDLR) elevates circulating levels of low-density lipoprotein cholesterol (LDL-C)

Surface receptor expression
Transports surface protein receptors for degradation in lysosomes (LDLR superfamily members, including LDLR, VLDLR, ApoER2, CD81, CD36, and ACE2)

Significance of the genetic locus

Located on the short arm of chromosome 1p32

Like LDLR and apolipoprotein B (APOB), the *PCSK9* gene is identified in familial hypercholesterolemia

Adjacent to the specific 1p34.1p32 locus correlated with augmented hepatic function to make cholesterol, connected to very low-density lipoprotein (VLDL), which transforms into LDL-C upon secretion

Mutant variants of PCSK9

Gain-of-function mutations/single nucleotide polymorphisms (SNPs)
- Elevated cholesterol levels
- Prevalence of coronary artery disease (CAD)

Heterozygote complete PCSK9 loss-of-function variants protect individuals from cardiovascular events (CVEs) and coronary heart disease (CHD) over a lifetime[1]

Loss-of-function mutations/SNPs
- Hypocholesterolaemia
- Cardiovascular protection

Y142X and C679X identified in African Americans showed 40% reduction in LDL-C and 88% lower incidence of CHD over 15 years[1]

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The role of PCSK9 in cardiovascular disease (CVD)

Lower blood cholesterol decreases the risk of developing atherosclerotic CVD (ASCVD), including stroke, myocardial infarction (MI), and CAD[2]

- PCSK9 variants that cannot be secreted from the endoplasmic reticulum cause hypercholesterolaemia[1]
- Serum LDL-C levels directly correlate with circulating PCSK9 levels[1]
- LDL–C lowering drugs or statins increase circulating PCSK9 levels
- Individuals with elevated PCSK9 levels exhibit increased LDL-C levels and early onset of CVEs

Molecular functions of PCSK9 in CVD[1,2]

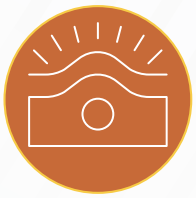
Normal LDLR recycling

- LDLRs on the hepatocyte cell surface bind to LDL-C in the blood
- LDLR–LDL-C complex is internalised within clathrin-coated vesicles with receptor-mediated endocytosis
- The acidic pH of endosomes induces LDLR to take on a closed conformation, releasing LDL-C[2]
- Dissociated LDL-C is degraded in the lysosomes
- LDLRs recycle back to the surface
- Removal of LDL-C from the circulation continues

PCSK9 impedes LDLR recycling

- Highly expressed in liver hepatocytes[2]
- The circulating form is captured by heparan sulphate proteoglycans and presented to LDL-R[2]
- Binds to LDLR–LDL-C on the hepatocyte cell surface
- Hinders LDLR conformation change
- Impedes LDLR recycling
- Promotes LDLR degradation, thereby increasing serum LDL-C levels[1,2]
- PCSK9–LDLR–LDL-C complex traffics to the lysosome for degradation

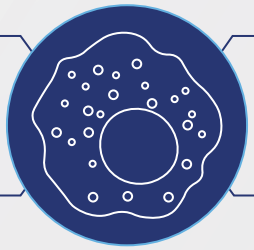
Role of PCSK9 in inflammation[1,2]



- Positively correlates with circulating levels of C-reactive protein and promote LDL-C uptake by residential macrophages in the artery
- Reactive oxygen species (ROS) generation and NOD-like receptor protein 3 (NLRP3) inflammasome signalling increase PCSK9 secretion
- Induces T helper 1 (Th1) and Th17 differentiation of naïve T lymphocytes, increasing the secretion of interferon-γ and interluekin-17A

Promotes foam cell formation resulting in atherosclerosis progression

PCSK9-LRP5 complex enhances TLR4/NF-κB signalling pathway, inducing the inflammatory process of atherosclerosis



Suppresses the anti-inflammatory action mediated by VLDL in human macrophages

Enhances *SRA, CD36,* and *LOX-1* gene and protein levels and elevates oxLDL-C uptake

Role of PCSK9 in thrombosis[2]

- Elevated level or enhanced functionality of PCSK9 advances atherosclerosis
- PCSK9 promotes platelet aggregation, activation, and expansion
- Interaction of PCSK9 with CD36 promotes thrombosis
- ROS-induced platelet activation leads to obstruction of microvessels and infarction
- PCSK9 induces neutrophil extracellular trap formation

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PCSK9 is a promising therapeutic target for CVD[1–3]

- PCSK9 deficiency significantly decreases LDL-C levels in circulation
- Lack of PCSK9 in animal models and human studies has no adverse effects (AEs)
- PCSK9-inhibitory therapies (PCSK9-iTs) target its synthesis, processing, and binding to prevent its molecular function during the onset of CVEs

Strategies

Monoclonal antibodies (mAbs)

- PCSK9 mAbs (evolocumab, alirocumab, and tafolecimab) block the interaction between PCSK9 and LDLR
- Evolocumab and alirocumab were approved by the European Medicines Agency (EMA) and the Food and Drug Administration of the United States (US FDA) in 2015
- Tofolecimab was approved in China in 2023

Small interfering RNAs (siRNAs)

- siRNA (inclisiran) selectively degrades *PCSK9* mRNA to suppress its translation
- Inclisiran was approved for clinical use by EMA in 2020 and the US FDA in 2021
- PCSK9-iTs significantly reduce blood LDL-C levels, decrease inflammatory markers, lower the risk of CVEs, and reduce platelet activation

Other PCSK9 inhibition strategies

Antisense oligonucleotide (ASO), small-molecule inhibitors, mimetic peptides, adnectin, anticalin, vaccines, CRISPR-based gene editing, and natural products

Current pharmaceutical strategies to target PCSK9[1]

	Mechanism of action	Advantages	Disadvantages	Administration	Candidates
mAbs	Block the PCSK9–LDLR interaction or neutralise PCSK9 activity	High specificity, low toxicity, efficient, safe	Frequent and parental administration, short shelf life, high cost	SI	Evolocumab, bococizumab, recaticimab alirocumab, tafolecimab, ebronucimab
siRNAs	Target *PCSK9* mRNA and inhibit translation	High specificity, infrequent dosing, long-term effect, safe	Parental injection	SI	Inclisiran
Antisense oligonucleotides SOs	Silence *PCSK9* mRNA	High specificity	High cost, parental administration	SI	AZD8233, BMS-844421, SPC5001
Small molecules	Block the synthesis and interaction of PCSK9 and enzyme	Oral administration, easy synthesis, low cost	Low selectivity, non-tissue specific effect	Oral	CVI-LM001, SAL092, DC371739
Mimetic peptides	Block PCSK9–LDLR interaction	High specificity, easy synthesis, low cost	Instable in plasma, parental administration	Oral or SI	MK-0616, Pep2-8
Adnectins	Block PCSK9–LDLR interaction	High specificity, easy synthesis, low cost	Short plasma half-life	SI or IV	Lerodalcibep, BMS-962476
Anticalin	Block PCSK9–LDLR interaction	Ab mimetic but smaller, low cost	Hard to design and screen	SI or IV	DS-9001a
Vaccines	Induce anti-PCSK9 autoantibodies	Long-term effect, infrequent dosing, low cost	Autoimmune disorder risk	SI or IM	AT04A, AT06A, VXX-401
Gene editing technology	Disrupt *PCSK9* gene	Infrequent dosing, long-term durable effect	Off-target potential, liver injury, integration into the genome	IV	Adenovirus-based, AAV-mediated, LLN-mediated
Natural products	Block the interaction and function of PCSK9 and enzyme or receptor	Oral administration, easy synthesis, low cost	Low selectivity, non-tissue specific effect	Oral	Berberine, monacolin K, curcumin, moracin C, polydatin

SI: Subcutaneous injection; IV: Intravenous

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Effect of PCSK9 inhibitory therapies[4,5]

- Lp(a) levels > ~50 mg/dL is an independent risk factor for ASCVD and a potential target for lipid-lowering therapy
- The African American community tends to have 2- to 4-fold higher Lp(a) levels than European Americans
- Conventional treatments with statins and other lipid-lowering therapies are not very effective

PCSK9 inhibition leads to Lp(a) reduction and greater coronary benefit

Alirocumab and evolocumab, result in 29.3% and 38.6% reduction from baseline in Lp(a) levels, respectively, at 24 weeks in phase 3 studies

Clinical trials for approved PCSK9-iTs against CVD[1]

FOURIER

- Evolocumab (SI, 140 mg biweekly or 420 mg monthly)
- 27,564 participants, (mean age: 62.5 years; 25% female)

Sustained decrease in LDL-C and decreased risk of recurring CVEs, with no AEs

ODYSSEY outcomes

- Alirocumab (SI, 75 mg biweekly)
- 18,924 participants, (mean age: 59 years; 25% female)

Reduced cholesterol and lowered the risk of ASCVD (MI, stroke, death) without AEs

The ORION program

A worldwide series of clinical studies

- Inclisiran (SI, single dose of 25–800 mg or multiple doses of 125–500 mg)

Decrease in both blood LDL-C and PCSK9 levels with a satisfactory safety feature

The CREDIT program

Encompasses various CREDIT trials

- Explores the therapeutic efficacy and clinical safety of tafolecimab (IBI306) among the Chinese population
- Tafolecimab (varying doses SI or IV)

Robust lipid-lowering efficacy in patients with hypercholesterolemia

Potential AEs associated with PCSK9 inhibitors[6]

Common AEs

- Injection-site reactions
- Influenza-like illness
- Myalgia

Uncommon AEs

- Hypersensitivity reactions
- Neurocognitive deterioration
- Musculoskeletal AEs

Key messages

- PCSK9 has been successfully targeted in CVD using many different strategies based on its varied biological roles
- Future clinical trials will focus on PCSK9 function in other disorders such as cancer and autoimmunity, with oral availability, efficacy, and long-term safety, as desired outcomes

References

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