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Review Article

Natural phytochemicals as small-molecule proprotein convertase subtilisin/kexin type 9 inhibitors

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Abstract

A decrease in the levels of low-density lipoprotein receptors (LDLRs) leads to the accumulation of LDL cholesterol (LDL-C) in the bloodstream, resulting in hypercholesterolemia and atherosclerotic cardiovascular diseases. Increasing the expression level or inducing the activity of LDLR in hepatocytes can effectively control hypercholesterolemia. Proprotein convertase subtilisin/kexin type 9 (PCSK9) protein, primarily produced in the liver, promotes the degradation of LDLR. Inhibiting the expression and/or function of PCSK9 can increase the levels of LDLR on the surface of hepatocytes and promote LDL-C clearance from the plasma. Thus, targeting PCSK9 represents a new strategy for developing preventive and therapeutic interventions for hypercholesterolemia. Currently, monoclonal antibodies are used as PCSK9 inhibitors in clinical practice. However, the need for oral and affordable anti-PCSK9 medications limits the perspective of choosing PCSK9 inhibitors for clinical usage. Emerging research reports have demonstrated that natural phytochemicals have efficacy in maintaining cholesterol stability and regulating lipid metabolism. Developing novel natural phytochemical PCSK9 inhibitors can serve as a starting point for developing small-molecule drugs to reduce plasma LDL-C levels in patients. In this review, we summarize the current literature on the critical role of PCSK9 in controlling LDLR degradation and hypercholesterolemia, and we discuss the results of studies attempting to develop PCSK9 inhibitors, with an emphasis on the inhibitory effects of natural phytochemicals on PCSK9. Furthermore, we provide insight into the mechanisms of action by which the reported phytochemicals exert their potential PCSK9 inhibitory effects against hypercholesterolemia.

Keywords: *Atherosclerotic cardiovascular diseases, Hypercholesterolemia, Low-density lipoprotein receptor, Proprotein convertase subtilisin/kexin type 9, Phytochemicals*

Introduction

Cholesterol is a critical component in the assembly of mammalian cell membranes, playing pivotal roles in intracellular functions such as organelle transport, signal transduction, and cell-microorganism interactions. Both endogenous synthesis and dietary absorption contribute to plasma cholesterol levels, with the liver being the primary site of cholesterol production. However, excessive synthesis can lead to hypercholesterolemia and dyslipidemia, posing significant health risks [1]. The low-density lipoprotein receptor (LDLR) pathway is a crucial route controlling cholesterol homeostasis [2]. In hepatocytes, lipids assemble with apolipoprotein B (ApoB)-100 to form very low-density lipoproteins (VLDLs), which are then secreted into the bloodstream. VLDL further metabolizes to form LDL, and cholesterol in the plasma mainly circulates within LDL (LDL cholesterol [LDL-C]). Most LDL-C in the bloodstream binds to LDLR on cell membranes, entering hepatocytes through endocytosis for metabolism. At the same time, peripheral cells express LDLR [3]. When LDLR activity is impaired, or its expression is reduced, plasma LDL-C cannot be transferred into hepatocytes for metabolism. This leads to LDL-C accumulation in circulation, and elevated LDL-C levels can result in hypercholesterolemia. Individuals with hypercholesterolemia are at risk of forming oxidized LDL, which attracts macrophages to form foam cells and is a critical factor in atherosclerosis. Therefore, individuals with

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hypercholesterolemia are at risk of developing atherosclerotic cardiovascular diseases (ASCVD), potentially leading to myocardial infarction (MI), stroke, or even sudden death [1,4]. Increasing the quantity of hepatic LDLR promotes the recycling of plasma LDL-C into hepatocytes, reducing plasma cholesterol levels.

Currently, statins are the primary drugs used clinically to treat hypercholesterolemia. Statins inhibit the critical enzyme HMG-CoA reductase (HMGCR) in the cellular cholesterol synthesis pathway. In recent years, the focus of treatment for hypercholesterolemia has shifted toward strategies aimed at increasing the quantity or activity of LDLR. Proprotein convertase subtilisin/kexin type 9 (PCSK9), a protein primarily responsible for promoting LDLR degradation in human hepatocytes, has become one of the target proteins for this purpose [5,6].

Molecular features of proprotein convertase subtilisin/kexin type 9

Characteristics of proprotein convertase subtilisin/kexin type 9 protein

Mutations in the LDLR, ApoB, and PCSK9 genes are linked to familial hypercholesterolemia (FH). Among FH patients, PCSK9 gene mutations occur in approximately 2.3% of cases. The PCSK9 gene is situated on chromosome 1p32, spanning 22 Kb, with 11 introns and 12 exons. The PCSK9 protein is newly synthesized as a 692-amino acid zymogen with a molecular weight of about 75 kDa [7]. While the liver is the primary site of PCSK9 expression, other organs such as the small intestine, kidney, and pancreas can also produce this protein.

The PCSK9 protein is a serine protease and a member of the proprotein convertase family in mammals [8]. Figures 1 and 2 illustrate the structural regions and biosynthesis processes of the PCSK9 protein. The structural components of the PCSK9 protein include the signal peptide (SP), prodomain (PD), catalytic domain (CD), hinge region, and the C-terminal cysteine-histidine-rich domain (CHRD). The CHRD region is divided into three repeated regions known as M1, M2, and M3. M1 and M3 play roles in the maturation and secretion of PCSK9, while M2 is involved in the intracellular degradation of LDLR [9,10]. In cells, the newly translated PCSK9 protein exists in a zymogen or proenzyme form, referred to as PreProPCSK9. The PreProPCSK9 protein loses its SP in the endoplasmic reticulum (ER) and becomes ProPCSK9 (~75 kDa). ProPCSK9 is then transported to the trans-Golgi network (TGN), undergoing autocatalytic cleavage. At the amino acid position, Q152, the PD (~15 kDa) is cleaved, and the mature PCSK9 (~62 kDa) is produced. During the transport and secretion of the active mature PCSK9 protein, the PCSK9 PD noncovalently binds to the mature PCSK9 protease chain (amino acids 153–692). This interaction prevents the binding of other proteins to the catalytic site. It inhibits the protease activity of PCSK9, keeping it in an inactivated state to allow continual transport into vesicles and secretion into the bloodstream [11]. PCSK9 has two forms in circulation: a mature PCSK9 (62 kDa) and a furin-cleaved PCSK9 (55 kDa). The furin-cleaved form is less active than the mature PCSK9 in degrading LDLRs [12].

Function of proprotein convertase subtilisin/kexin type 9 protein

Figure 3 illustrates the function of PCSK9, primarily focused on facilitating the degradation of LDLR within the lysosomes of liver cells. This degradation process occurs through two distinct pathways: the extracellular and intracellular pathways [9]. PCSK9 binds to LDLR on the cell surface in the extracellular pathway, triggering LDLR degradation. Typically, LDLR undergoes recycling; however, on LDL binding to LDLR, endocytosis is initiated, leading to the formation of endosomes. Within endosomes, the lower pH causes the separation of LDL from LDLR. LDL undergoes lysosome metabolism, while LDLR returns to the cell membrane [13].

Recently, Coppinger *et al*. reported the crystal structure detailing the outer interactions between PCSK9 and LDLR [14]. The CD of secreted mature PCSK9 in the

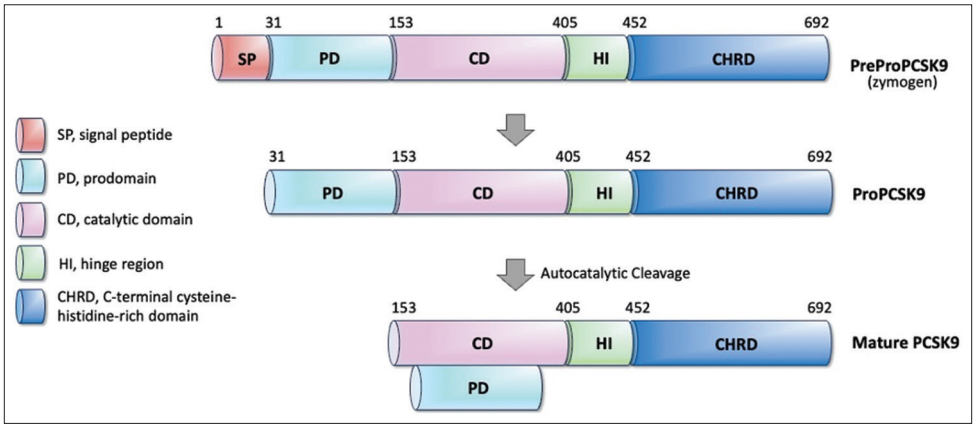

Figure 1: The structural regions of proprotein convertase subtilisin/kexin type 9 (PCSK9) protein. The PreProPCSK9 protein consists of the signal peptide (SP, amino acids 1–30), (prodomain, amino acids 31–152), (catalytic domain, amino acids 153–404), hinge region, amino acids 405–452, and the (C-terminal cysteine-histidine-rich domain, amino acids 452–692). Upon losing its SP in the endoplasmic reticulum, the PreProPCSK9 protein transforms into ProPCSK9, which then undergoes autocatalytic cleavage to yield the mature PCSK9. PCSK9: Proprotein convertase subtilisin/kexin type 9, SP: Signal peptide, PD: Prodomain, CD: Catalytic domain, CHRD: C-terminal cysteine-histidine-rich domain

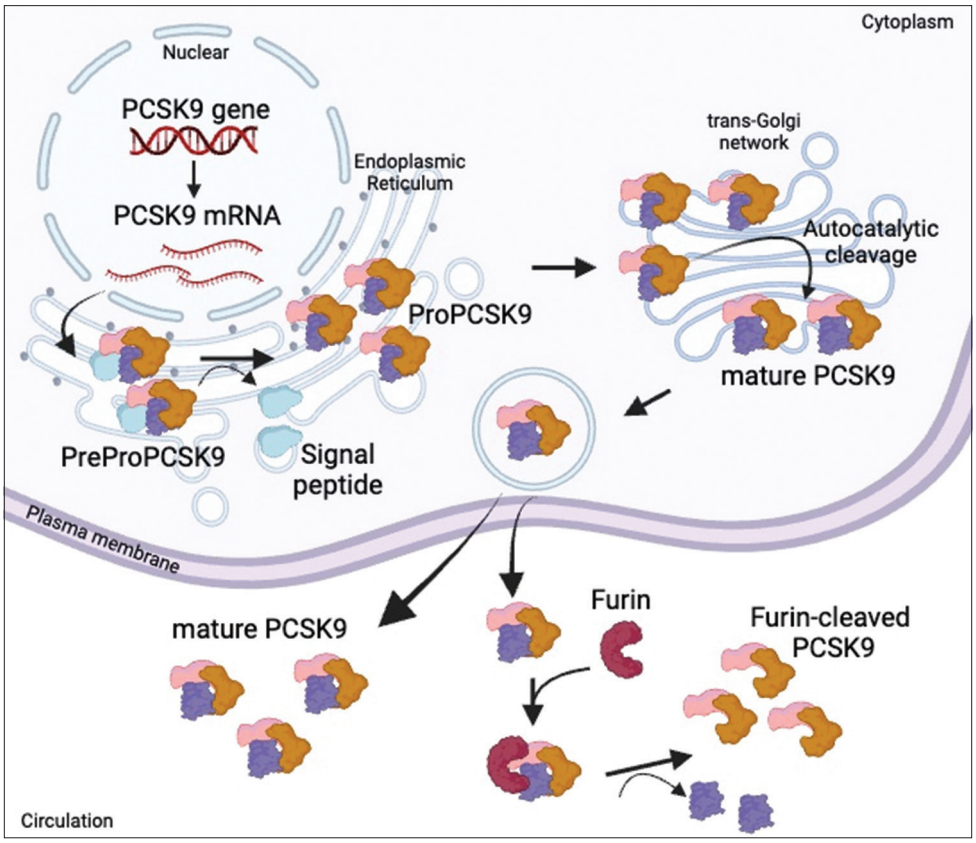

Figure 2: The biosynthesis of the proprotein convertase subtilisin/kexin type 9 (PCSK9) protein. The PCSK9 protein initially translated as PreProPCSK9, a zymogen form, which loses its signal peptide in the endoplasmic reticulum to become ProPCSK9. ProPCSK9 is then transported to the trans-Golgi network where it undergoes autocatalytic cleavage, producing mature PCSK9. In the bloodstream, PCSK9 exists in two molecular forms: a mature PCSK9 form and a furin-cleaved PCSK9 form (Created with BioRender.com). PCSK9: Proprotein convertase subtilisin/kexin type 9

extracellular space binds to the epidermal growth factor A (EGF-A) region of LDLR. This interaction predominantly occurs within a hydrophobic amino acid region spanning 1000 Å. The binding of PCSK9 to LDLR is calcium dependent and enhanced with decreasing pH value. The PCSK9-LDLR complex undergoes endocytosis, entering the cell to form endosomes. The lower pH within endosomes boosts PCSK9's affinity for LDLR, facilitating tight binding. Subsequently, this complex is directed to lysosomes for degradation, preventing LDLR from recycling to the cell surface [15,16]. Consequently, the number of LDLRs on the cell membrane diminishes, reducing the absorption of plasma LDL-C into the liver for breakdown. In the intracellular pathway, the PCSK9 protein directly binds to LDLR in the TGN, redirecting it to lysosomes for degradation before it reaches the cell surface. This leads to a reduction in LDLR quantity and, consequently, a decrease in LDLR presence on the cell surface [17].

Impact of proprotein convertase subtilisin/kexin type 9 in hypercholesterolemia and atherosclerotic cardiovascular diseases

When PCSK9 binds to LDLR before LDL particles and LDLR enter liver cells, it triggers premature degradation of LDLR. This reduction in the number of LDLRs on the cell surface limits LDL-C transport into the liver for breakdown. Consequently, blood LDL-C levels rise, contributing to the development of hypercholesterolemia, a significant risk factor for ASCVD. Studies using model animals have demonstrated that mice with heightened PCSK9 expression exhibit elevated plasma LDL levels due to reduced LDLR levels [18]. Experiments showed that in PCSK9$^{-/-}$mice, increased LDLR expression in liver cells was observed [19]. Furthermore, PCSK9 promotes the degradation of hepatic LDLR, resulting in increased levels of ApoB-containing lipoprotein particles and VLDL triglycerides (TG), consequently leading to hypertriglyceridemia. Conversely, PCSK9 deficiency resulted in reduced TG levels [20]. Studies on humans revealed that individuals with loss-of-function mutations in the PCSK9 gene have lower plasma LDL levels and a decreased risk of coronary heart disease, coronary artery bypass surgery, or MI. Conversely, individuals with gain-of-function mutations in the PCSK9 gene exhibit higher plasma LDL-C levels. These findings suggest that PCSK9, which promotes hepatic LDLR degradation, not only influences cholesterol balance but also impacts TG balance, leading to dyslipidemia and ASCVD [21,22]. Consequently, PCSK9 has become a crucial clinical research target for treating hypercholesterolemia. Efforts have been made to reduce the levels or inhibit the function of PCSK9 through drugs or supplements. These efforts aim to decrease PCSK9 binding to LDLR, thereby reducing LDLR degradation, leading to an increased number of LDLRs in the liver and decreased blood LDL-C levels, ultimately lowering the risk of ASCVD [23].

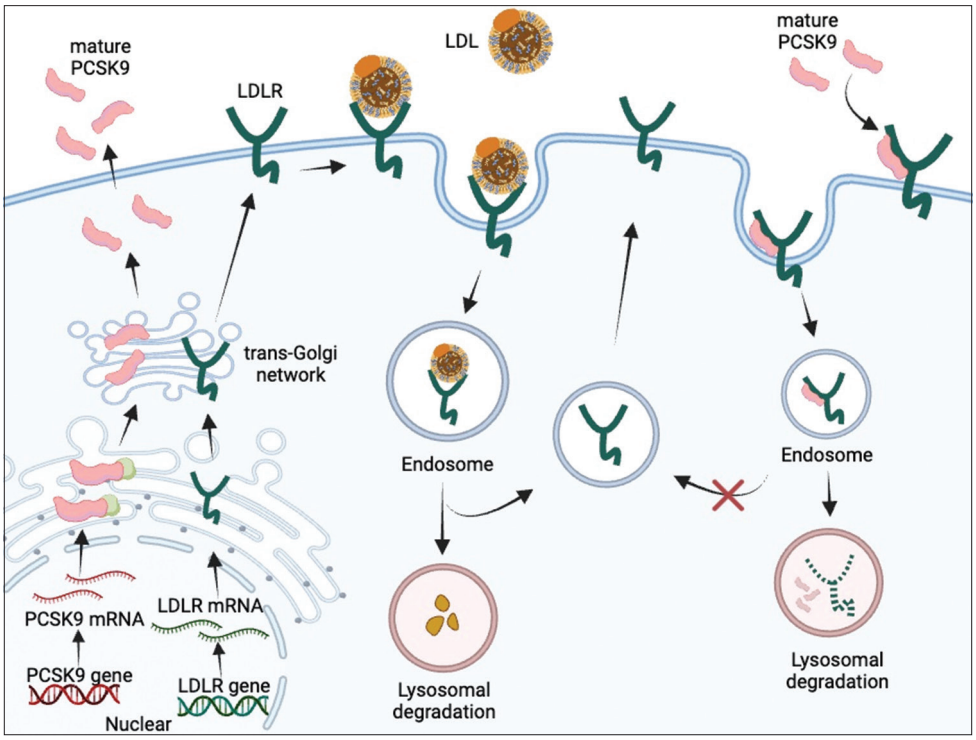

Figure 3: The function of proprotein convertase subtilisin/kexin type 9 (PCSK9) in low-density lipoprotein receptor (LDLR) degradation. The process begins with the binding of LDL to LDLR, initiating endocytosis and forming endosomes. Within endosomes, the lower pH causes LDL to separate from LDLR. LDL undergoes metabolism in lysosomes, while LDLR returns to the cell surface. Meanwhile, PCSK9 binds to LDLR on the cell surface, initiating endocytosis and entering the cell to form endosomes. The lower pH within endosomes increases the binding affinity of the PCSK9-LDLR complex. Subsequently, this complex is directed to lysosomes for degradation, impeding LDLR from recycling to the cell surface (Created with BioRender.com). PCSK9: Proprotein convertase subtilisin/kexin type 9, LDL: Low-density lipoprotein, LDLR: Low-density lipoprotein receptor

Pharmacological inhibition of proprotein convertase subtilisin/kexin type 9

Inhibiting PCSK9 function increases the quantity of LDLR in liver cells, enhancing LDL-C clearance from the bloodstream. Therefore, developing drugs that inhibit PCSK9 activity, thus preventing LDLR degradation, has become a focus of developing novel strategies for preventing and treating hypercholesterolemia and ASCVD. Additionally, studies have indicated that adults carrying PCSK9 deficiency genes with low PCSK9 expression levels do not exhibit clinical symptoms, and their blood LDL-C levels are low, suggesting that the inhibition of PCSK9 function may not cause adverse effects on human health [24,25]. Hence, developing PCSK9 inhibitors is feasible and holds significance for treating hypercholesterolemia [26]. Several strategies have been proposed for inactivating PCSK9, including monoclonal antibodies (mAbs), antisense oligonucleotides, gene editing by CRISPR, and small molecules [Figure 4].

Proprotein convertase subtilisin/kexin type 9 inhibitors

PCSK9 is currently targeted by two FDA/EMA-approved PCSK9 mAb inhibitors: evolocumab and alirocumab [27]. These mAbs represent the sole available anti-PCSK9 treatments, functioning by binding to free plasma PCSK9, thereby preventing its interaction with LDLR on hepatocytes. This action increases the recycling of LDLR and significantly reduces circulating LDL-C, consistent with the mechanism mentioned above [26]. PCSK9 mAbs hold the potential for managing cholesterol by targeting the PCSK9 protein, but their use in humans poses limitations and health risks [13,28]. Their large protein size results in high development and manufacturing costs. Administering injections every 2–4 weeks inconveniences patients and may trigger long-term immune reactions. Side effects of PCSK9 mAb inhibitors include injection site reactions, flu-like symptoms, myalgia, potential neurocognitive effects, back pain, and heightened risk of musculoskeletal and connective tissue disorders [29,30]. The long-term impacts on liver function and the immune system are also still being investigated. Hence, health-care providers and patients should thoroughly assess the potential benefits of PCSK9 mAb inhibitors against these established and possible risks before usage. Moreover, these inhibitors are solely used as therapeutics and cannot be used for preventive health care. In recent years, an alternative to anti-PCSK9 mAbs has emerged as the PCSK9 siRNA inhibitor inclisiran, designed to target PCSK9 mRNA in the liver [31]. However, PCSK9 siRNA faces drawbacks such as a longer pharmacokinetic curve, requirement of injection administration, and unresolved safety concerns [32]. Like mAbs, PCSK9 siRNA inhibitors are restricted to therapeutic use and cannot be used preventively.

Recently, small molecules have emerged as a promising avenue in PCSK9 inhibition. These molecules require lower costs and techniques compared to mAb and siRNA inhibitors. As they can be developed for oral administration, they provide greater convenience and can be employed for therapeutic and

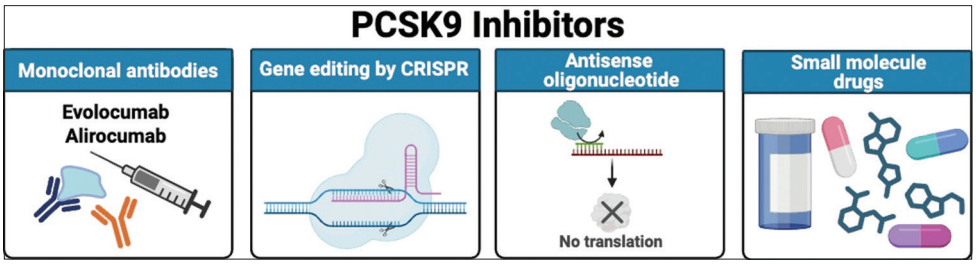

Figure 4: Development strategies for proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors. Various strategies have been developed for the pharmacological inhibition of PCSK9, including monoclonal antibodies like evolocumab and alirocumab, the CRISPR genome editing technology, and antisense oligonucleotides for treating hypercholesterolemia. Developing oral and low-cost small-molecule inhibitors is a novel pharmacological approach for PCSK9 inhibition (Created with BioRender.com). PCSK9: Proprotein convertase subtilisin/kexin type 9

preventive purposes. Consequently, developing small-molecule PCSK9 inhibitors has become an important research focus.

Approaches to developing small-molecule inhibitors targeting proprotein convertase subtilisin/kexin type 9

The inhibition mechanisms of PCSK9 can be categorized into three major types [14]:

(a) Inhibitors of PCSK9 gene expression: This approach aims to reduce PCSK9 gene expression, decreasing PCSK9 protein synthesis. In this category, PCSK9 inhibitors are primarily developed as PCSK9 siRNA, PCSK9 CRISPR (gene editing) [33], and small molecules that inhibit PCSK9 transcription and translation [34]. An increasing number of small molecules are under investigation for inhibiting PCSK9 transcription and translation.

(b) Inhibitors of PCSK9-LDLR binding: This approach prevents the interaction between PCSK9 and LDLR, allowing more LDLR to be recycled to the cell surface for LDL-C clearance. In this category, PCSK9 inhibitors are mainly developed as mAbs, peptides, vaccines, and small molecules [35]. Research on small molecules for PCSK9 inhibition through this mechanism is limited due to their small-molecular weight (<500 Da), making it challenging to disrupt the PCSK9-LDLR binding [36]. Monoclonal antibodies and peptide drugs remain the primary designs for inhibitors using this mechanism

(c) Inhibitors of PCSK9 autocatalytic activity and maturation process: This approach blocks the self-cleavage activity of ProPCSK9, hindering PCSK9 protein maturation and secretion [37]. This approach reduces the amount of mature PCSK9 in the bloodstream. In this category, small molecules are particularly suitable, and although research in this field has only just begun, developing small-molecule PCSK9 inhibitors targeting this mechanism should be promising in the near future.

Developing proprotein convertase subtilisin/kexin type 9 small-molecule inhibitors using molecular modeling

Currently, there is no clinical application of small molecules for PCSK9 inhibition, making the development of such inhibitors a novel, significant, and viable strategy. Investigation into the binding abilities of small molecules to proteins and identifying binding sites on proteins can be carried out with computational molecular docking analysis [38]. Petrilli *et al*. conducted molecular docking analysis to study the binding between PCSK9 and small molecules [39], and the results revealed a predicted binding pocket between the CD and the CHRD of the human PCSK9 protein. Moreover, a study on co-crystallization of the small molecule and PCSK9 protein and X-ray crystallography analysis confirmed that the small-molecule compound could indeed bind to the predicted binding pocket of PCSK9. This study indicated that applying molecular docking to predict the binding of small molecules to the PCSK9 protein is a feasible strategy for screening and developing PCSK9 small-molecule inhibitors.

Research perspectives on phytochemical-based natural proprotein convertase subtilisin/kexin type 9 inhibitors

Many small-molecule compounds with health-promoting and disease-treating activities originate from natural phytochemicals. Numerous studies have suggested that phytochemicals obtained from daily intake of vegetables, fruits, or herbal sources contribute positively to human health. Polyphenolic phytochemicals, particularly flavonoids, are abundant in the diet and found in vegetables, fruits, tea, and coffee, known for their antioxidant properties [40]. Flavonoids have been found to have various biological activities, such as antioxidant, anti-inflammatory, and anti-cancer effects. They share a typical core structure of a C6-C3-C6 ring, and the biological activities of flavonoids often depend on the different functional groups of the ring structure. Flavonoids play a crucial role in preventing and treating cardiovascular diseases, diabetes, cancer, and neurodegenerative disorders [41,42]. Substantial evidence supports regulating cellular processes, transcription factors, signaling pathways, epigenetics, and enzymatic activities by natural phytochemicals, ultimately influencing lipid metabolism and reducing the risk of ASCVD [43].

Mechanisms of action of phytochemical-based proprotein convertase subtilisin/kexin type 9 inhibitors

Figure 5 illustrates the natural phytochemicals as PCSK9 inhibitors currently under investigation and their mechanisms of action. Studies on the most common mechanisms of phytochemical PCSK9 inhibition primarily focus on reducing PCSK9 gene expression by inhibiting its transcription. Examples include curcumin, berberine, resveratrol, pinostrobin, fisetin, kaempferol, naringin, and

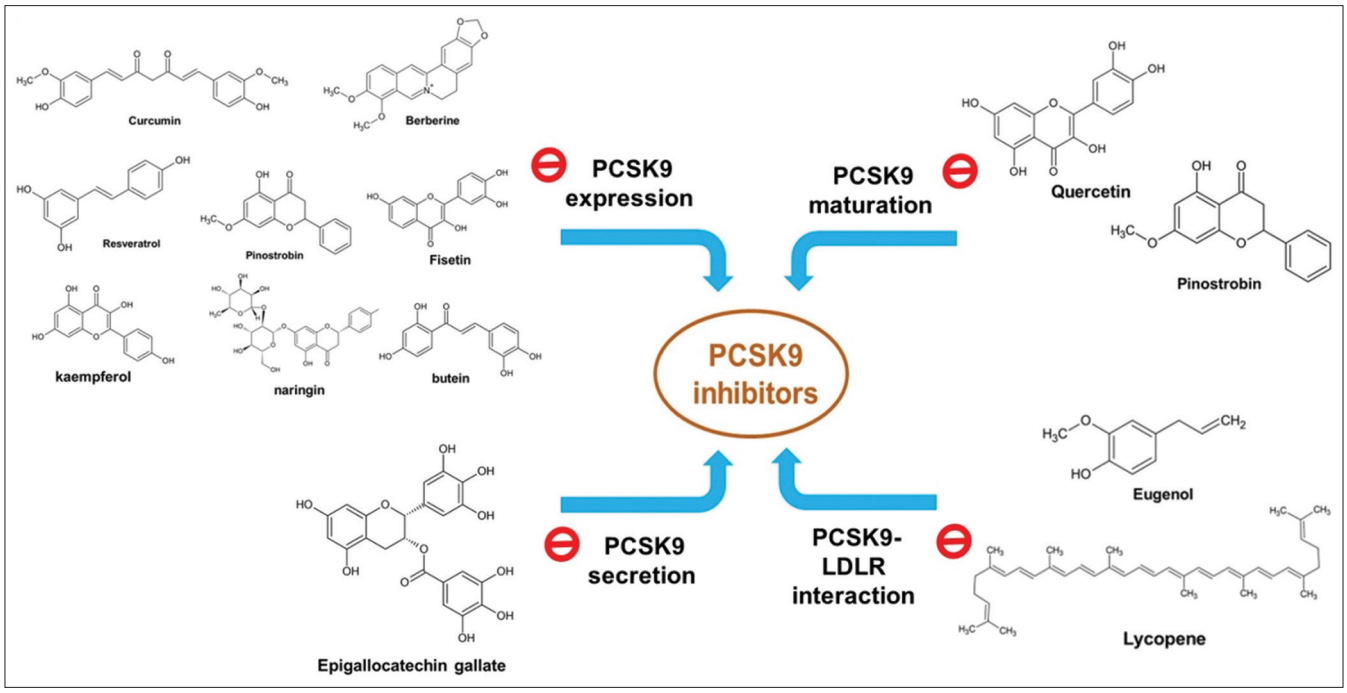

Figure 5: Mechanisms of action in the investigation of phytochemical-based proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors. Research on natural phytochemical PCSK9 inhibitors mainly focuses on inhibiting PCSK9 gene expression. Examples include curcumin, berberine, resveratrol, pinostrobin, fisetin, kaempferol, naringin, and butein. Other compounds like eugenol and lycopene affect the PCSK9-low-density lipoprotein receptor interaction. Few, like quercetin and pinostrobin, act on PCSK9 protein maturation, while epigallocatechin gallate influences PCSK9 secretion. PCSK9: Proprotein convertase subtilisin/kexin type 9, LDLR: Low-density lipoprotein receptor

butein. Other compounds, such as eugenol and lycopene, affect the interaction between PCSK9 and LDLR. Only a few, such as quercetin and pinostrobin, act on the PCSK9 protein maturation process, while epigallocatechin gallate (EGCG) influences PCSK9 secretion from cells. Although only limited phytochemical PCSK9 inhibitors have been reported in the literature, this review summarizes current available studies on these compounds [Table 1], which will be discussed in the following few sections.

Nonflavonoid polyphenols as proprotein convertase subtilisin/kexin type 9 inhibitors

Curcumin

Curcumin, a significant bioactive polyphenolic phytochemical extracted from the rhizome of *Curcuma longa*, has been studied for its potential health benefits. Tai *et al*. reported that curcumin (at concentrations of 10 and 20 μM) notably reduced PCSK9 gene expression by inhibiting the activity of the nuclear transcription factor HNF1α and enhancing LDL uptake in HepG2 cells. Furthermore, curcumin was observed to counteract the PCSK9-inducing effects of lovastatin, indicating its potential as a novel addition to cholesterol-lowering combination therapy [44]. However, it is worth noting that the concentrations of curcumin used in *in vitro* cell systems were higher than those typically achievable *in vivo*, which may limit the relevance of these findings. In a study by Cai *et al*., it was demonstrated that treatment with curcumin (at a dosage of 200 mg/kg/day) for 12 weeks in cirrhotic rats inhibited hepatic PCSK9 expression, leading to increased LDLR levels that could potentially improve LPS detoxification [45]. Nonetheless, curcumin's poor solubility, stability, and bioavailability under physiological conditions present challenges, limiting its therapeutic utility and necessitating careful examination through appropriate pharmacological investigations in humans [46].

Berberine

Berberine, the primary active phytochemical found in Berberis species' roots, rhizomes, and stem bark, has garnered attention for its potential health benefits. Cameron *et al.* reported that berberine reduces PCSK9 mRNA and protein levels in HepG2 cells. In addition, combining berberine with mevastatin mitigated increased PCSK9 mRNA levels induced by statins while boosting LDLR mRNA and protein levels in hepatic cells [47]. Li *et al*. demonstrated that berberine (at 20 μM) markedly suppresses PCSK9 transcription through a modest reduction in the transcription factors HNF1α and SREBP2 in HepG2 cells [48]. Dong *et al*. showed that berberine (at doses of 200 mg/kg/day for mice and 100 mg/kg/day for hamsters) reduces circulating PCSK9 protein and hepatic PCSK9 mRNA levels by modulating HNF1α levels in hyperlipidemia mice and hamsters. They suggested that berberine treatment might promote the degradation of the HNF1α protein through the ubiquitin-proteasome degradation pathway in hepatic cells [49]. Ma *et al*. reported that berberine (at doses of 50 and 100 mg/kg/day) significantly improves dyslipidemia, mitigates aortic plaque formation, and reduces hepatic lipid accumulation in ApoE$^{-/-}$mice fed a high-fat diet. These effects were associated with downregulating hepatic PCSK9 expression by activating the ERK1/2 pathway [50]. Despite the favorable effects of berberine on lipid metabolism, its intestinal absorption *in vivo* is often minimal. Therefore, enhancing the bioavailability

Table 1: Research perspectives on phytochemical-based natural proprotein convertase subtilisin/kexin type 9 inhibitors

Phytochemicals	Models	Mechanisms of action	References
Nonflavonoids polyphenols			
Curcumin	HepG2 cells	Inhibition of PCSK9 expression via regulating HNF-1α	[44]
	Cirrhotic rats	Inhibition of hepatic PCSK9 expression	[45]
Berberine	HepG2 cells	Inhibition of PCSK9 mRNA and protein expression	[47]
	HepG2 cells	Inhibition of PCSK9 expression via regulating HNF-1α and SREBP2	[48]
	Hyperlipidemia mice and hamsters	Inhibition of hepatic PCSK9 expression via regulating HNF-1α	[49]
	High-fat diet-fed ApoE$^{-/-}$ mice	Inhibition of hepatic PCSK9 expression via activating ERK1/2	[50]
Resveratrol	Hepatic steatosis L02 cells	Inhibition of PCSK9 expression	[54]
	Ovx/ApoE$^{-/-}$ mice	Inhibition of hepatic PCSK9 expression	[55]
	Insulin-treated HepG2 cells	Inhibition of PCSK9 expression via regulating ER-α	[55]
EGCG	HepG2 cells	Reduction of secreted PCSK9 protein	[57]
Lycopene	Hyperlipidemia rats	Inhibition of hepatic PCSK9 mRNA expression	[58]
	High-fat diet-fed rats	Inhibition of hepatic PCSK9 expression	[59]
	In silico molecular docking	Interaction with PCSK9 for affecting PCSK9 - LDLR interaction	[59]
Eugenol	*In silico* molecular docking	Interaction with PCSK9 for affecting PCSK9 - LDLR interaction	[63]
Flavonoids			
Quercetin	Huh7 cells	Inhibition of PCSK9 maturation via regulating sortilin	[64]
	High-cholesterol diet-fed mice	Inhibition of PCSK9 expression	[65]
	Raw264.7 macrophages	Inhibition of PCSK9 expression	[66]
Pinostrobin	HepG2 cells	Inhibition of PCSK9 expression via activating FOXO3	[68]
	HepG2 cells	Inhibition of PCSK9 self-catalytic cleavage and maturation	[68]
Fisetin	High-fat diet-fed ApoE$^{-/-}$ mice	Inhibition of PCSK9 expression	[69]
Kaempferol	HepG2 cells	Inhibition of PCSK9 expression via regulating SREBP2	[71]
Naringin	Obese C57BL/6J mice	Inhibition of PCSK9 expression via regulating AMPK and SREBP2	[72]
Butein	HepG2 cells	Inhibition of PCSK9 expression via regulating HNF-1α	[73]

PCSK9: Proprotein convertase subtilisin/kexin type 9, LDLR: Low-density lipoprotein receptors, EGCG: Epigallocatechin gallate, ER: Estrogen receptor

of berberine is crucial, and it may involve synthesizing derivatives and employing nanotechnology approaches for drug delivery [51,52].

Resveratrol

Resveratrol, a bioactive polyphenol derived from *Veratrum grandiflorum*'s roots and commonly found in red wine, grape seeds, and peanuts, has attracted attention for its potential health benefits [53]. In a hepatic steatosis cell model, resveratrol (at concentrations of 10 and 20 μM) has been shown to decrease PCSK9 expression, elevate LDLR protein levels, and enhance LDL uptake, suggesting its significant role in alleviating nonalcoholic fatty liver disease [54]. In the liver of Ovx/ApoE$^{-/-}$mice, resveratrol (at doses of 250 and 500 mg/kg) reduces PCSK9 expression, consequently increasing LDLR levels and the uptake of LDL in hepatocytes. In addition, inhibition of to estrogen receptor α (ERα) activity abolishes the resveratrol-mediated decrease in PCSK9 in insulin-stimulated HepG2 cells. These findings indicate that resveratrol may potentially hinder postmenopausal atherosclerosis progression by downregulating PCSK9 expression through the ERα-mediated signaling pathway [55]. However, resveratrol is a lipophilic compound prone to photosensitivity, with poor solubility and limited bioavailability *in vivo* [56]. Currently, there is a lack of data regarding the cholesterol-lowering effects of resveratrol on the regulation of PCSK9 expression in humans, highlighting the need for further research to explore this aspect.

Epigallocatechin gallate

EGCG, the primary polyphenolic catechin in green tea, has garnered interest for its potential health benefits. Kitamura *et al*. found that EGCG (at 25 μM) reduces the secretion of PCSK9 protein from HepG2 cells while increasing LDLR levels. Despite decreasing extracellular PCSK9 protein, EGCG did not affect PCSK9 mRNA or intracellular precursor/mature PCSK9 levels. Moreover, EGCG was observed to mitigate the inducing effect of lovastatin on PCSK9 protein secretion [57]. Several studies have suggested an association between green tea consumption and hypocholesterolemia. However, evidence regarding the impact of EGCG on PCSK9 levels in humans is lacking, highlighting the need for further investigations to understand better the bioactivity of EGCG concerning PCSK9.

Lycopene

Lycopene, a lipid-soluble carotenoid abundant in fruits and vegetables, particularly in tomatoes and tomato-based products, has emerged as a subject of interest for its potential health benefits. In an *in vivo* study, treatment with lycopene (at 10 mg/kg body weight/day) reduced hepatic PCSK9, HMGR, and HNF-1α mRNA expression, subsequently upregulating LDLR through the activation of SREBP-2 in hyperlipidemia rats [58]. In a rat model fed a high-fat diet, lycopene (at doses of 5, 10, and 50 mg/kg body weight/day) significantly decreased the expression of hepatic PCSK9 and HMGCR genes, leading to a notable increase in hepatic LDLR expression. In addition, lycopene mitigated the impact of inflammation-induced PCSK9 expression by inhibiting the expression of tumor necrosis factor-α, interleukin (IL)-1 β, and IL-6. Results from *in silico* molecular docking analysis affirmed that lycopene can occupy the active site of the PCSK9 protein crystal structure, thus diminishing the affinity of PCSK9 to bind to the EGF-A

domain of LDLR. Consequently, this interference with PCSK9 function promotes LDLR degradation and improves hypercholesterolemia [59]. Despite its cholesterol-lowering effects, the clinical applications of lycopene remain limited due to its low bioavailability. This challenge can be addressed through nanotechnology or by consuming processed tomatoes with added olive oil. Many aspects of lycopene's metabolism, function, and clinical adaptation in the human body still require clarification. Nevertheless, incorporating lycopene-rich products into the diet can be an effective preventive measure against cardiovascular disease [60].

Eugenol

Eugenol, a prominent phenolic compound in the essential oil of clove, displays cholesterol-modulating properties. Animal studies have revealed that eugenol reduces plasma cholesterol levels and diminishes hepatic lipogenesis, suggesting potential benefits in addressing atherosclerosis and fatty liver disease [61,62]. Moreover, recent molecular docking analysis has unveiled hydrophobic interactions between eugenol and the PCSK9 protein [63]. This finding suggests that a physical interplay between these molecules could influence the interaction between PCSK9 and LDLR, offering insights into potential mechanisms underlying eugenol's effects.

Flavonoids-based phytochemicals as proprotein convertase subtilisin/kexin type 9 inhibitors

Quercetin

Quercetin is a flavonoid present in several fruits and vegetables. Mbikay *et al.* found that quercetin-3-glucoside (concentration range from 1 to 10 μM) reduced PCSK9 mRNA expression by 20%–30% in Huh7 cells. The compound increased intracellular PCSK9 protein levels by 20%–90% but reduced PCSK9 secretion into the extracellular medium by 30%–35%. This effect was attributed to quercetin-3-glucoside's negative regulation of the protein sortilin, which induces PCSK9 secretion from the trans-Golgi network to the cell membrane [64]. In a high-cholesterol diet-fed mouse model, quercetin-3-glucoside (0.05 and 0.1% w/w) regulated PCSK9 and LDLR, subsequently reducing abnormal blood lipid levels [65]. In addition, quercetin suppressed the expression of PCSK9 not only in hepatic cells but also in macrophages. These findings support quercetin's anti-atherogenic and anti-inflammatory effects through the downregulation of PCSK9 expression in macrophages [66,67]. The results indicate that the flavonoid quercetin can potentially influence both PCSK9 gene expression and the maturation process of PCSK9, demonstrating its impact on lipid metabolism.

Pinostrobin

Pinostrobin, a bioactive flavanone found in honey and plants such as *Pinus strobus*, *Cajanus cajan*, and *Boesenbergia pandurata*, has been studied for its potential influence on PCSK9. Gao *et al*. discovered that pinostrobin (20 and 40 μM) significantly reduces PCSK9 mRNA and protein expression dose dependently by activating FOXO3 in HepG2 cells. In addition, pinostrobin interferes with PCSK9 self-catalytic cleavage activity and inhibits PCSK9 maturation, increasing LDLR expression and LDL uptake by HepG2 cells [68].

Fisetin

Fisetin is mainly found in strawberries and exhibits health-promoting activities, including the regulation of hyperlipidemia. Yan *et al*. reported that fisetin (12.5 mg/kg body weight) inhibited the PCSK9 expression and improved atherosclerosis in apoE$^{-/-}$mice fed a high-fat diet [69]. Guo *et al*. discovered that fisetin (30 μM) promotes the gene expression of cholesterol efflux transporters ABCG5/G8 and ABCB1 while inhibiting the expression of the cholesterol uptake regulator NPC1L1 in Caco-2 cells. In addition, fisetin increases the protein expression of LDLR and decreases the expression of PCSK9 [70].

Other flavonoids

The influence of a few other flavonoid compounds on PCSK9 has also been demonstrated in *in vivo* or *in vitro* experimental systems. Choi *et al*. found that Welsh onion extract, possibly through the flavonoid kaempferol, inhibits PCSK9 gene expression by suppressing SREBP2 functionality [71]. Sui *et al*. demonstrated in a mouse study that naringin, a flavonoid in citrus, activated the AMPK pathway, leading to decreased SREBPs and PCSK9 expression, increased LDLR expression, and subsequently reduced liver cholesterol and TG, as well as body weight [72]. Hwang *et al*. discovered that butein, by reducing HNF-1α expression, decreased PCSK9 mRNA expression and extracellular PCSK9 levels in HepG2 cells [73]. These results suggest that flavonoids can regulate PCSK9 and its associated physiological functions.

Conclusions

Currently, statins are the only small-molecule compounds that can be taken orally to treat high cholesterol levels. Statins primarily inhibit cholesterol synthesis in hepatocytes, but they are known to cause side effects such as muscle pain. For some patients with high cholesterol levels, the therapeutic effectiveness of statins is suboptimal. Moreover, statins may also promote an increase in PCSK9, which could reduce the efficacy of statins and increase the risk of ASCVD. In clinical practice, PCSK9 inhibitors have been employed to treat patients who do not respond well or cannot be treated with statins. Although the mechanisms of action differ between PCSK9 inhibitors and statins in terms of inhibition of cholesterol biosynthesis, the focus of research is shifting toward using PCSK9 inhibitors to reduce blood cholesterol levels. However, the current strategies for this purpose primarily involve the application of PCSK9 mAbs. These strategies currently need more cost-affordable and orally applicable small-molecular drugs. To solve this problem, developing phytochemical-based PCSK9 inhibitors might offer alternative and feasible therapeutic options to statin treatments. These phytochemical compounds may also be used with statins, benefiting patients with lipid metabolism disorders. Understanding the binding pockets of the PCSK9 protein using molecular simulation analysis helps develop phytochemical inhibitors of PCSK9. The rational design of small molecules as orally administered PCSK9 inhibitors may thus serve as novel pharmacological approaches for treating hypercholesterolemia and ASCVD.

Data availability statement

The data-sharing statement is not applicable to this article since no datasets were generated or analyzed during the current study.

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Conflicts of interest

There are no conflicts of interest.

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