Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy Dovepress
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REVIEW

Therapeutic targets of hypercholesterolemia: HMGCR and LDLR

This article was published in the following Dove Press journal:
Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy

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Abstract: Cholesterol homeostasis is critical and necessary for the body's functions. Hypercholesterolemia can lead to significant clinical problems, such as cardiovascular disease (CVD). 3-Hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) and low–density lipoprotein cholesterol receptor (LDLR) are major points of control in cholesterol homeostasis. We summarize the regulatory mechanisms of HMGCR and LDLR, which may provide insight for new drug design and development.

Keywords: cholesterol homeostasis, CVD, HMGCR, LDLR

Introduction

Cholesterol plays a key role in the regulation of the body's essential functions. It is both one of the basal components of biological membranes and a precursor of a variety of physiologically active substances, such as bile acid, vitamin D, steroid hormones, ubiquinol and heme A, which are intermediate products of mevalonic cholesterol biosynthesis and exhibit pleiotropic effects on numerous diseases and energy metabolism.[1–6] The main source of the body's cholesterol is from mevalonic biosynthesis (de novo synthesis), particularly in the liver, where up to 1 g cholesterol can be synthesized, and in the extrahepatic tissues such as the small intestines and adrenal glands.[7–11] 3-Hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) is the rate-limiting enzyme in the de novo synthesis of cholesterol and serves as a key regulatory enzyme controlling endogenous cholesterol synthesis.[11,12]

About 500 mg of total cholesterol is consumed each day in the bile acid synthesis pathway in the liver for lipid digestion and absorption, and 400–500 mg of dietary exogenous cholesterol is absorbed from the intestines each day.[3,9,10] Additionally, hepatic cholesterol is transported in the blood by lipoproteins and utilized by peripheral tissues (Figure 1).[13] The serum cholesterol is mainly metabolized through the low-density lipoprotein cholesterol (LDL-c) receptor (LDLR) pathway.[14] In the peripheral tissues, LDLR on the cell surface membranes binds to plasma LDL-c particles transporting liver cholesterol, and LDL-c is then taken in by endocytosis and cleared.[12,15]

Hypercholesterolemia can lead to significant clinical problems such as cardiovascular disease (CVD), and hypocholesterolemic agents significantly reduce the risk of CVD events.[16–22] Therefore, as key regulatory points in cholesterol metabolism, HMGCR and LDLR have been studied as targets for treating CVD and dyslipidemia in recent decades.[23,24] In this review, we summarize the regulatory mechanisms of HMGCR and LDLR, which may provide insights for new drug design and development.

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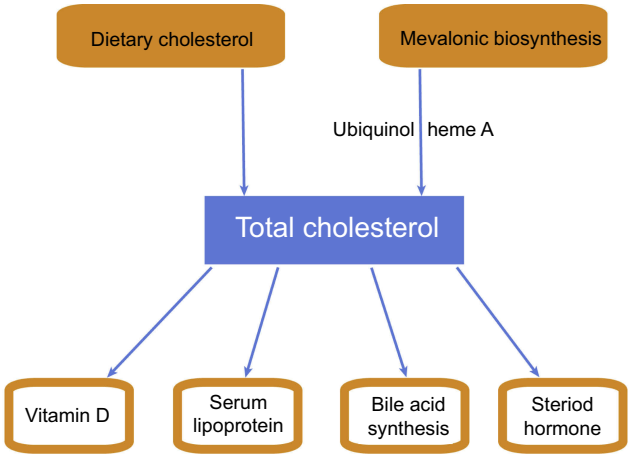

Figure 1 Schematic diagram of cholesterol metabolic pathway in the body.

Regulatory mechanism of HMGCR

The human HMGCR gene (GeneID: 3156) is located on chromosome 5q12,[25] and it encodes three isoforms (isoforms 1, 2 and 3) that are produced by alternative splicing and may lead to different responses to treatment with statin (an HMGCR inhibitor).[26] HMGCR isoform 1 has been extensively investigated and is a membrane-bound glycoprotein comprising 888 amino acids that regulates mevalonate, which is an initial control point in the endogenous biosynthesis of cholesterol in the liver and small intestine.[27] The activity and amount of HMGCR are regulated at multiple levels and through multiple mechanisms, such as negative feedback regulatory mechanisms mediated by sterols and nonsterol metabolites derived from mevalonate, post-translational modification, degradation and hormone regulation.[11,27–33] Different regulatory pathways interact to control cholesterol homeostasis (Figure 2).

Negative feedback regulation

Negative feedback regulation is the most important way to control cholesterol synthesis. The cholesterol synthesis process is very complex, involving over 30 enzymatic reactions. HMGCR, the primary rate-limiting enzyme in the process, is a known target of feedback regulation whose concentration directly influences the amount of cholesterol synthesized.[34] An increase in the concentration of cholesterol or 25-hydroxycholesterol (25-OH cholesterol) suppresses the synthesis of HMGCR and leads to a marked decrease in HMGCR; furthermore, cholesterol accelerates HMGCR degradation by facilitating HMGCR ubiquitination. These two mechanisms result in a synergistic effect, ultimately leading to a decrease in both the HMGCR concentration and cholesterol biosynthesis to decrease the concentration of cholesterol.[28,30,31,35] The

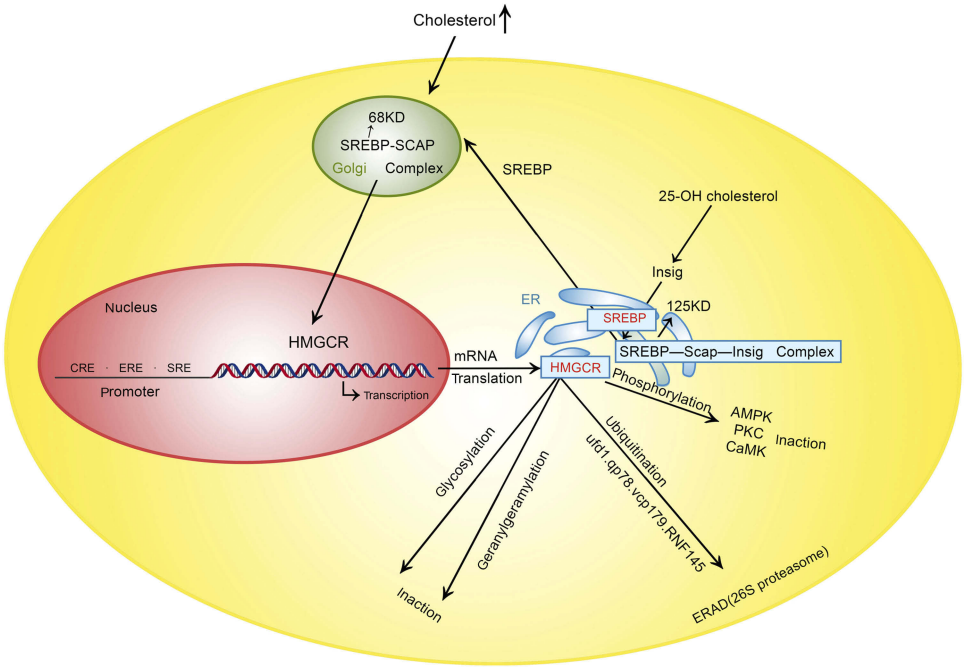

Figure 2 Control point of HMGCR.

Abbreviations: HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; SRE, Sterol regulatory element; ER, endoplasmic reticulum; SREBP, sterol regulatory element binding protein; SCAP, SREBP cleavage activating protein; AMPK, AMP-activated protein kinase; PKC, Protein kinase C; CaMK, Ca2+/calmodulin-dependent protein kinase; ERAD, ER-associated degradation; 25-OH cholesterol, 25-hydroxycholesterol; RNF145, ring finger protein 145.

suppression of HMGCR synthesis is associated with a type of nuclear transcription factor termed sterol regulatory element-binding proteins (SREBPs).[36]

SREBPs, including SREBP-1a (GeneID: 6720) and SREBP-1c (GeneID: 6720), which are produced from the same gene by alternative splicing, and SREBP-2 (GeneID: 6721), are key lipogenic transcription regulators. SREBP-1a is a potent activator of all SREBP-responsive genes, and SREBP-1c activates genes involved in fatty acid and triglyceride synthesis. SREBP-2 preferentially regulates genes involved in the cholesterol synthesis pathway. SREBP-2 activates the transcription of genes by binding sterol regulatory element 1 (SRE-1; 5′-ATCACCCCAC-3′) in the LDLR and HMGCR promoters.[36]

SREBP-2 is initially formed as a 125 kDa precursor in the endoplasmic reticulum (ER), and its nuclear mature form (68 kDa) can enter the nucleus to activate the transcription of target genes only after proteolytic processing (which involves removal of its carboxyl terminus in the Golgi apparatus). In the Golgi apparatus, SREBP can interact with SREBP cleavage activating protein (SCAP). SCAP can combine with a pair of ER membrane protein insulin-induced genes (Insig1 and Insig2) to form the SREBP/SCAP/Insig complex. This complex becomes fixed on the ER after combining with Insig.[36–38] SCAP (GeneID: 22937) contains 1279 amino acids and possesses a sterol-sensing domain (SSD);[39] cholesterol can bind to the SSD of SCAP and thus change the conformation of SCAP. The SREBP/SCAP complex is retained in the ER when the sterol concentration is high, leading to the suppression of SREBP-mediated transcription and HMGCR product. When the cholesterol in cells is depleted, SCAP dissociates from the SREBP/SCAP/Insig complex and SREBP enters the Golgi complex to be processed, and the synthesis of cholesterol subsequently increases due to SREBP-mediated transcription.[38,40–42] There is an obvious distinction between 25-OH cholesterol and cholesterol in the feedback regulation of cholesterol synthesis, and a recent study demonstrated that 25-OH cholesterol inhibits the synthesis of cholesterol by binding to Insig, not SCAP, although cholesterol is thought to bind to SCAP.[38] Recent evidence demonstrated that sulfation of 25-OH cholesterol might inhibit lipid synthesis and inflammatory responses, and it may be a target for CVD prevention.[43,44]

Mevalonate-derived products participating in the feedback regulation of cholesterol synthesis are closely associated with the ER-associated degradation (ERAD) of HMGCR and can act synergistically with sterols to augment HMGCR degradation by facilitating both the ubiquitination of HMGCR and its dislocation out of the ER, which is involved in the geranylgeranylation of proteins (geranylgeranyl pyrophosphate (GGPP).[45] The prevention of geranylgeranylation may be another mechanism by which statins lower cholesterol.[46,47]

Posttranslational modification of HMGCR

Importantly, the posttranslational modification of HMGCR influences its regulatory function, which depends on cellular energy conditions. Two forms of HMGCR exist: phosphorylated (inactive) and dephosphorylated (active) forms.[33,48–50] The phosphorylation of human HMGCR at serine 872 by AMP-activated protein kinase (AMPK) reduces its enzymatic activity, and the dephosphorylation of HMGCR by protein phosphatase restores its enzymatic activity.[48,51] Metformin, the most widely used hypoglycemic drug that acts by activating AMPK, can regulate lipid metabolism.[52–54] Protein kinase C (PKC) and Ca^{2+}/calmodulin-dependent protein kinase (CaMK) are also involved in the phosphorylation of HMGCR, but the specific phosphorylated residue in HMGCR has not been identified.[55,56]

The ubiquitination of HMGCR mainly influences its stability and facilitates its degradation to regulate cholesterol production. When cellular sterol accumulates, HMGCR binds to Insig1 and gp78, which is an E3 ubiquitin ligase, and interacts with ATPase valosin-containing protein (VCP/p97) in the ER to facilitate the ubiquitination (at lysine 248) and ERAD of HMGCR by the cytosolic 26S proteasome,[31,57,58] while Ufd1, a gp78 cofactor, enhances and accelerates the ERAD of HMGCR.[35] In liver-specific gp78 knockout mice, SREBP was decreased and this was accompanied by elevated levels of Insig1/2, leading to decreased cholesterol synthesis. However, the degradation of HMGCR was decreased, suggesting that gp78 may play an important role in the degradation of SREBP.[59] Small-molecule compounds have shown a beneficial therapeutic effect in dyslipidemia by inhibiting the SREBP pathway, which suggests that SREBP may be a new drug target in the future.[60,61] Ring finger protein 145 (RNF145) is a ubiquitin ligase involved in the degradation of HMGCR that was recently identified based on small-scale short hairpin RNA (shRNA) screening, and its cysteine 537 residue is critical for its function.[62] Glycosylation is also involved in the negative regulation of HMGCR activity, which induces HMGCR localization to the ER.[63] Therefore, another way to control cholesterol

metabolism is by influencing the level of posttranslational modification of HMGCR.

Hormone regulation and genetic polymorphisms

The HMGCR promoter region contains a cyclic AMP response element (CRE) and an estrogen response element (ERE) in addition to a sterol regulatory element (SRE). These elements are involved in the regulation of HMGCR transcriptional activity.[64,65] Cellular cholesterol levels are vital for the regulation of glial cell development and myelination by neuregulin, and the control of cholesterol synthesis by neuregulin was shown to be partly mediated by a CRE sequence in the HMGCR promoter.[64] Estrogen transactivated HMGCR expression via binding to the ERE (AGTCCcatCGACC) in the HMGCR promoter, which induced an elevation in the total cholesterol and LDL-c levels in the newborns of pregnant women with high estradiol levels.[65]

Studies have shown that genetic polymorphisms of HMGCR are associated with its function and phenotype. Akadam-Teker et al have demonstrated that the total cholesterol and LDL-c levels are higher among male coronary heart disease patients aged <55 years carrying the HMGCR CC genotype (rs3761740) than those carrying CA + AA genotypes, which indicates an association between HMGCR polymorphisms and CVD.[66] However, the A allele of the HMGCR genotype increased the risk of Alzheimer's disease (AD) in an Italian study.[67] Conversely, the rs3761740 variant of HMGCR was not associated with AD in a Swedish case-control study.[68] Another HMGCR polymorphism (rs3846662) may increase the mRNA and protein levels of HMGCR by affecting alternative splicing, and thereby contribute to the onset and progression of AD.[69–72] Additionally, gene polymorphisms affect the cholesterol-lowering response to statin treatment.[73–75]

Regulatory mechanism of LDLR

LDLR, which is the receptor for LDL-c, plays a critical role in cholesterol transport and clearance from the plasma to the cytoplasm, and its functional insufficiency can lead to familial hypercholesterolemia (FH) and increase the risk of CVD.[76–80] LDLR functioning is regulated through various means, such as genetic variants, feedback regulation associated with gene transcription, posttranslational modifications and degradation (Figure 3). LDLR knockout mice are a well-established model of atherosclerosis that aid clinical research into the treatment of human atherosclerosis.[53,81–84]

Genetic variants

The LDLR gene (Gene ID: 3949), located on chromosome 19p13.2, encodes six isoforms produced by alternative splicing. The canonical sequence (isoform 1) contains 860 amino acids but undergoes glycosylation in the ER and is changed into a mature 839 amino acid form in the Golgi apparatus, which is then transported to the cell membrane.[78,85,86] LDLR includes an extracellular domain (amino acids 22–788), a transmembrane domain (amino acids 789–810) and a cytoplasmic domain (amino acids

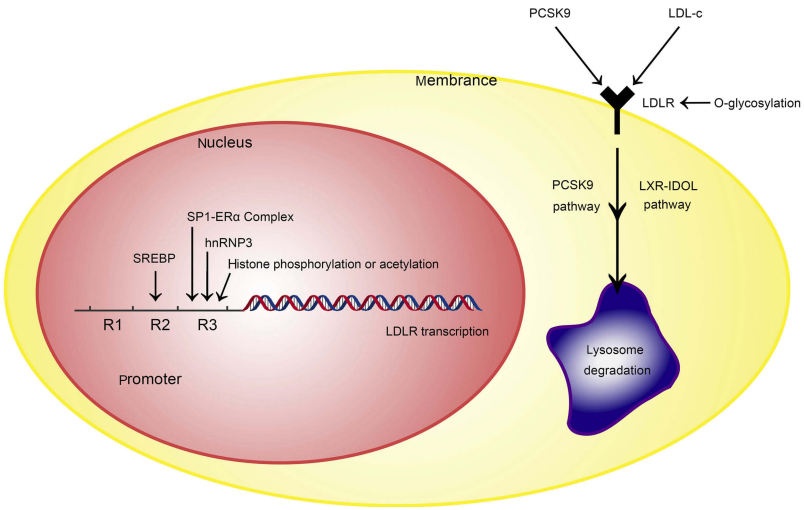

Figure 3 Control point of LDLR.
Abbreviations: LDLR, low–density lipoprotein cholesterol receptor; SREBP, sterol regulatory element binding protein; IDOL, inducible degrader of the LDLR; PCSK9, proprotein convertase subtilisin/kexin type 9; LXR, Liver X receptors; R1, repeat 1 sequence; R2, repeat 2 sequence; R3, repeat 3 sequence; ERα, estrogen receptor α.

811–860). Natural variants and mutations of the LDLR gene have been reported to influence its function and were involved in FH, which was characterized by high plasma LDL-c levels, dyslipidemia and CVD.[87–90] For example, a single-nucleotide variation (rs767618089) of the LDLR gene at position 300 in the protein sequence of the extracellular domain did not affect LDLR expression but resulted in a reduction in LDL-c binding activity and LDL-c uptake.[91] A mutation at position 828 in the cytoplasmic domain did not affect binding activity but reduced LDLR internalization.[92] To date, more than 2000 LDLR genetic variants have been described, and many have not been demonstrated to have pathological significance.[87,93,94] Interestingly, there is a decreased risk of type 2 diabetes in patients with FH,[95,96] but these findings need to be investigated for the further prevention of diabetes.

Feedback regulation and SREBP-2

LDLR expression is controlled by the negative feedback regulation of intracellular cholesterol via the SREBP-2 pathway due to an SRE-1 sequence in the LDLR promoter.[36,97–101] When the cellular sterol level is low, LDLR expression is transcriptionally stimulated by nuclear SREBP and the specific transcription factor Sp1, which binds to the *cis*-acting element (repeat 3 sequence, R3) of the LDLR promoter with a synergistic effect.[102,103] Sp1 phosphorylation at Thr453 and Thr739 is critical for Sp1 binding to the LDLR promoter to stimulate LDLR expression.[103] Additionally, Sp1 is required for the increase of LDLR transcription by 17β-estradiol, in which 17β-estradiol binds to the estrogen receptor α (ERα)/Sp1 complex to *trans*-activate LDLR promoter activity in HepG2 cells.[104,105] The heterogeneous nuclear ribonucleoprotein K (hnRNP K) protein, a heterogeneous nuclear ribonucleoprotein, controls LDLR transcriptional activity by specifically interacting with R3 in the LDLR promoter, which is also the binding site of Sp1, though the relationship between hnRNP K and Sp1 remains unknown.[101]

Posttranslational modification of LDLR

The regulation of LDLR also involves histone modifications and extensive posttranslational modifications that influence the stability or activity of nucleic acids and functional proteins. PKC induced the phosphorylation of histone H3 Ser10 at the LDLR promoter and stimulated the expression of LDLR.[106] In addition, the level of histone acetylation at the LDLR promoter affected its transcriptional activity.[101]

N- and O-glycosylation play crucial roles in protein processing from the Golgi apparatus to the ER, which influences membrane protein folding and stability, cell signal transduction, ligand binding, immunological defense and organ development.[107–109] The ligand-binding domain of LDLR is O-glycosylated, which increases its affinity for LDL-c by ~5-fold.[108,109]

The ubiquitination of a lysine residue is related to the degradation of LDLR depending on inducible degrader of the LDLR (IDOL), which is an E3 ubiquitin ligase, in a liver X receptor (LXR)-induced regulatory manner. IDOL catalyzes the polylysine 63-linked ubiquitination of LDLR, thereby promoting LDLR lysosomal degradation and decreasing LDL-c clearance, and this regulatory process is not dependent on the SREBP-2 pathway.[110–114] Studies of IDOL-knockout mice have reported an improvement in metabolic dysfunction, including a decrease in circulating cholesterol, triglyceride and glucose levels and hepatosteatosis and fat mass; these effects suggest that the inhibition of IDOL may be a future therapeutic strategy to combat dyslipidemia and/or CVD.[115,116]

Proprotein convertase subtilisin/kexin type 9 (PCSK9) regulatory pathway and LDLR degradation

PCSK9, a secretory serine protease, is involved in the degradation of LDLR.[117–119] Ten phosphorylated serine residues have been identified on LDLR based on high-throughput screening using mass spectrometry, but it is not clear whether these phosphorylated serine residues are related to PCSK9.[120,121] PCSK9 facilitated LDLR degradation in lysosomes by binding to ligand-binding repeats in the LDLR extracellular domain and led to a dramatic increase in plasma LDL-c, which was reversed by the loss of PCSK9 function and other anti-PCSK9 strategies.[118,119] Alirocumab and evolocumab, novel PCSK9 inhibitors, exhibited a beneficial effect and improved dyslipidemia without an increase in diabetes risk in clinical studies.[24,122–127] PCSK9 is also a downstream protein of SREBP-2 because of the SRE sequence in the PCSK9 promoter.[128] A recent study showed that triciribine, a specific AKT inhibitor, inhibited PCSK9 expression by SREBP-2 transcriptional regulation accompanied by a decrease in HMGCR expression. In addition, triciribine induced LDLR mRNA stability and increased LDLR protein levels in

a phosphorylated AKT-extracellular signal-regulated kinase (ERK)-dependent manner. Therefore, triciribine exerts beneficial and overlapping hypocholesterolemic effects, which make it an attractive potential drug target for hypercholesterolemia prevention.[129]

LDLR gene transcription was activated by the proinflammatory cytokine oncostatin M (OM), which induced a 3.8-fold maximal increase in LDLR mRNA levels in HepG2 cells, and this effect was mediated by the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) and mitogen-activated protein kinase 1 (MEK1)/ERK-PCSK9 pathways.[130–132] OM downregulated PCSK9 transcription, which was accompanied by an elevation in LDLR and a significant decrease in plasma total cholesterol.[130] The next works need to elucidate whether these findings have an important implication for future drug development. Studies demonstrated that an OM-induced complex involving CCAAT/enhancer binding protein (c/EBP), cAMP-responsive element binding protein (CBP) and early growth response gene 1 (Egr1) was required to bind to the sterol-independent regulatory element (SIRE) in the PCSK9 promoter and suppress PCSK9 expression.[133–135] Different transcriptional factors were recruited and interacted as a complex to regulate downstream gene expression, and the complex also involved PKC or other regulators, demonstrating complex regulation.[136]

Perspectives

Intracellular cholesterol homeostasis depends on the balance between supply, including the intestinal intake of dietary cholesterol and its intracellular synthesis, and demand, including the bile acid pathway, the synthesis of steroid hormones and the clearance of cholesterol by the low-density lipoprotein (LDL)-LDL receptor (LDLR) pathway. Hypercholesterolemia, an imbalanced and pathologic state of cholesterol homeostasis, is a major risk factor for cardiovascular disease (CVD), which is the leading cause of mortality worldwide.[14,137] Up to now, the ezetimibe and bile acid sequestrants as second-line cholesterol lowering agents were used to inhibit the cholesterol intake from the intestine. Up to now, the ezetimibe and bile acid sequestrants as second-line cholesterol lowering agents were used to inhibit the cholesterol intake from the intestine.[138,139] The 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors (statins, alirocumab and evolocumab) have been widely used to treat hypercholesterolemia in clinical settings. These four classes of cholesterol-lowering agents acts in different ways (Figure 4), but clinical studies demonstrated that these drugs need to be improved to achieve more effective outcomes.[140,141] Therefore, for hypercholesterolemia and CVD prevention, further

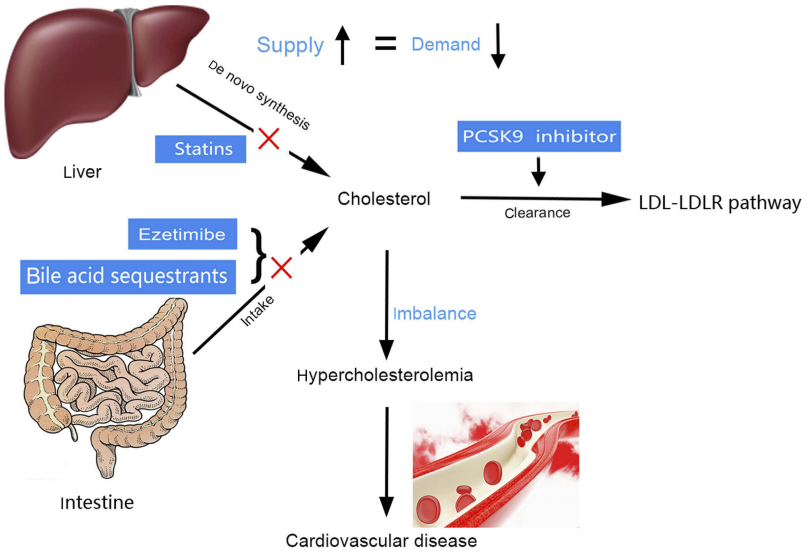

Figure 4 Schematic diagram of the hypercholesterolemia and current therapeutic drug. PCSK9 inhibitor: proprotein convertase subtilisin/kexin type 9 inhibitor.
Abbreviations: LDL, low–density lipoprotein; LDLR, low–density lipoprotein cholesterol receptor.

research is necessary to develop specific agents targeted at the HMGCR and LDLR regulatory pathway.

Acknowledgments

This work was funded from the project ZR2019BH023 supported by Shandong Provincial Natural Science Foundation and National Natural Science Foundation of China (grant number:81900716). The sponsors had no role in any of the stages from study design to submission of the manuscript for publication.

Disclosure

The authors report no conflicts of interest in this work.

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