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Low-Density Lipoprotein Receptor (LDLR) Family Orchestrates Cholesterol Homeostasis

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The LDLR family of proteins is involved in lipoproteins trafficking. While the role of LDLR in cardiovascular disease has been widely studied, only recently the role of other members of the LDLR proteins in lipoprotein homeostasis and atherosclerosis has emerged. LDLR, VLDLR, and LRPs bind and internalize apoE- and apoB-containing lipoprotein, including LDL and VLDL, and regulate their cellular uptake. LRP6 is a unique member of this family for its function as a co-receptor for Wnt signal transduction. The work in our laboratory has shown that LRP6 also plays a key role in lipoprotein and TG clearance, glucose homeostasis, and atherosclerosis. The role of these receptor proteins in pathogenesis of diverse metabolic risk factors is emerging, rendering them targets of novel therapeutics for metabolic syndrome and atherosclerosis. This manuscript reviews the physiological role of the LDLR family of proteins and describes its involvement in pathogenesis of hyperlipidemia and atherosclerosis.

INTRODUCTION

Lipoprotein transport of cholesterol in plasma plays a physiological role for essential energy production, cell membrane, and hormone synthesis. Cells readily utilize cholesterol by internalizing lipoprotein ligands containing chylomicron, low-density lipoprotein (LDL\textsuperscript{†}), intermediate-density lipoprotein (IDL), or very low-density lipoprotein (VLDL) mediated by the LDL

\textsuperscript{†}Abbreviations: ACAT, acyl-CoA cholesteryl acyl transferase; ARH, autosomal recessive hypercholesterolemia protein; EGF, epidermal growth factor; FH, familial hypercholesterolemia; GSK-3, glycogen synthase kinase-3; HDL, high-density lipoprotein; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; IDL, intermediate-density lipoprotein; LEP, lymphoid enhancer factor; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor; LPL, lipoprotein lipase; LRP, low-density lipoprotein related protein; MESD, mesoderm development; PCSK9, proprotein convertase subtilisin/kexin type 9; PDGF, platelet-derived growth factor; PKC, protein kinase C; PPAR, peroxisome proliferator-activated receptor; SREBP, sterol regulatory element-binding protein; TCF, T cell factor; TG, triglycerides; VLDL, very low-density lipoprotein; VLDLR, very low-density lipoprotein receptor; VSMC, vascular smooth muscle cells.

Keywords: cardiovascular disease, LDL clearance, LDLR family, lipoprotein trafficking
receptor (LDLR) family of membrane receptors. Internalized lipoprotein particles undergo an endocytic process that includes clustering of lipoprotein receptors in coated pits, transport to early and late endosomes followed by hydrolysis in lysosomes, release of the lipid to cytoplasm, and recycling of receptors back to cell surface. LDLR-mediated endocytosis has provided much of our understanding of lipoprotein clearance, and its defect is a major cause of familial hyperlipidemia and a fundamental risk factor for cardiovascular disease.

Elevated LDL is a prevalent risk factor for coronary artery disease and other atherosclerosis diseases, which constitute the largest cause of all mortalities in the United States [1]. Defect in LDLR is the most common cause of familial hypercholesterolemia (FH) and premature coronary artery diseases. The role of LDLR in cardiovascular diseases has been extensively studied. However, only recently the role of other members of the LDLR family of proteins in lipoprotein homeostasis and atherosclerosis has emerged [2-5]. While significant advances have been made in understanding the balance between cholesterol synthesis and transport, the mechanisms surrounding cholesterol homeostasis and hypertriglyceridemia remain incompletely understood. Therefore, this review will provide basic information on the role of the LDLR family in lipid homeostasis and pathogenesis of cardiovascular disease.

**LDLR FAMILY**

The LDLR family comprises a group of endocytic receptors on the cell surface, which bind and internalize extracellular ligands, including lipoproteins, exotoxins, and lipid-carrier complexes [6]. Members of the LDLR
family are structurally and functionally related to LDLR, which is the patriarch of the entire family. Proteins of the LDLR family share structurally common motifs (Figure 1): LDLR type A repeats, epidermal growth factor (EGF)-like domain, transmembrane anchor, and, in certain instances, cytoplasmic domain. LDLR and VLDLR additionally contain α-link sugar domain, located just outside of the plasma membrane. LRP1 and LRP2 (megalin) have relatively large extracellular domains [7]. LDLR type A repeats are localized to a region at NH2-terminal and responsible for binding of ligands, including apoB-100- and apoE-containing lipoprotein. EGF-like precursor contains multiple EGF repeats along with a β-propeller domain and is involved in pH-dependent dissociation of ligand-receptor complex. Transmembrane domain helps anchor the receptors to the membrane. Cytoplasmic domain contains NPxY (Asn-Pro-any amino acid (x)-Tyr)-containing domain or PPPSP motif (Pro-Pro-Pro-Ser-Pro) [7] and is involved in the targeting of receptors to coated pits and signal transduction. Differences in position and number of each domain create the diversity in LDLR family members. To date, numerous members of the LDLR family are reported that participate in a wide range of physiological processes [8]. In particular, LDLR, VLDLR, LRP5/6, LRP1, and LRP2 play a pivotal role in cholesterol homeostasis and lipid metabolism [9-13].

**LDLR**

LDLR is a cell membrane glycoprotein that functions in the binding and internalizing of circulating cholesterol-containing lipoprotein particles. LDLR is ubiquitously expressed and is a key receptor for main-
taining cholesterol homeostasis in mammals. LDLR-mediated endocytosis is essential for lipoprotein and lipid metabolism [14] (Figure 2). The clearance of LDL, a major carrier of cholesterol in human, by LDLR is extensively studied [14-19]. Recognition of apoB-100 of LDL particles occurs with a stoichiometry of a single copy of apoB-100 per one LDL particle per receptor monomer [20]. VLDL, IDL, high-density lipoprotein (HDL), and chylomicron remnant are also recognized by LDLR at neutral pH [21,22]. Receptor-ligand complexes undergo endocytosis via clathrin-coated pits. Coated vehicle dispenses to endosomes with LRP6 and autosomal recessive hypercholesterolemia protein (ARH, also known as LDLR adaptor protein), connecting the LDLR family protein and the endocytic machinery; thereby, acidic condition activates dissociation of internalized ligands. Released ligand particles further travel to the lysosome, in which the ligand is degraded by enzyme, while the receptors recycle back to the cell surface. LDLR contains seven LDLR type A repeats in binding domain, immediately followed by EGF-like modules, transmembrane anchor, and NPxY-repeats containing cytoplasmic domain. LDL particles trigger three steps after internalization: 1) reducing gene expression of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) to suppress cholesterol biosynthesis; 2) enhancing activity of acyl-CoA cholesteryl acyl transferase (ACAT) to reduce toxic free cholesterol; and 3) suppressing LDLR synthesis to reduce LDL uptake via SREBPs [23-25].

Impaired LDLR function by genetic mutations results in a condition with extremely elevated serum LDL levels and early onset atherosclerosis known as familial hypercholesterolemia (FH) [26]. The prevalence is 1:500 for heterozygote and 1 in a million for homozygote FH [27]. While normal fibroblasts have high cell surface binding affinity for LDL via LDLR, cultured fibroblasts from FH patient fail to clear serum LDL, thus causing elevated serum LDL [16,19]. Patients homozygote for LDLR mutation have serum LDL levels that are as high as 800 mg/dL and show widespread accumulation of cholesterol-mediated atheromatous plaques in their coronary arteries, aorta, and aortic valves [18]. Heterozygote LDLR mutation carriers have typically twice-elevated plasma LDL concentration and two-fold increase risk for coronary artery disease compared to the general population. Most guidelines suggest adjustment of LDL levels to less than 100 mg/dL in individuals with two or more risk factors for coronary artery disease and 70 mg/dL for patients with established coronary artery disease. Approximately 50 percent of heterozygote FH patients develop various forms of cardiovascular disease in the fourth or fifth decades [17]. LDLR mutations can be classified into five groups based on the functional characteristics of the encoded proteins: 1) null alleles causing receptor synthesis-defect; 2) transport-defective alleles with defect in targeting receptor to cell surface; 3) binding-defective alleles that encode proteins that fail to bind ligands; 4) internalization-defective alleles that encode proteins that fail to interact with clathrin coated pit; and 5) recycling-defective alleles, which remain undissociated in the acidic pH of lysosomes [28].

Ldlr<sup>-/-</sup> mice fed a 1.5 percent high cholesterol diet not only exhibit enhanced hyperlipidemia and increased generation of reactive oxygen species, but also alter vascular structure along with increased collagen content [29,30]. LDLR gene transfer by intravenous injection of adenovirus to Ldlr<sup>-/-</sup> mice reduces plasma LDL and VLDL cholesterol [10]. Autosomal recessive hypercholesterolemia clinically resembles FH but is caused by mutations in an LDLR adaptor protein, which is named after the disorder (ARH). LDLR adaptor protein deficient mice (Arh<sup>-/-</sup>) have impaired LDLR internalization in a variety of tissues such as the liver and exhibit a major defect in LDL clearance [31,32]. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a protein secreted from the hepatocytes, which undergo protein-protein interaction with LDLR, resulting in their degradation [33]. Gain of function in humans causes hypercholesterolemia, and loss of function is associated with low LDL chole-
terol and protection against atherosclerosis. RNAi targets PCSK9, specifically lower plasma LDL cholesterol, by preventing LDLR degradation in vivo and in vitro [34]. Similarly, inhibition of PCSK9 effectively increases hepatic LDLR expression and reduces LDL cholesterol in plasma in mice [35]. In sum, LDLR is a pivotal receptor for endocytic machinery and plays a pivotal role in maintaining cholesterol homeostasis. Defect in LDLR function or expression triggers elevated LDL cholesterol and results in major atherosclerotic diseases.

**VLDLR**

VLDLR, along with LDLR and LRP1, is the main endocytic receptor recognizing apoE-containing lipoproteins. VLDLR is widely expressed in adipose tissues, skeletal muscle, heart, and endothelial cells of capillaries and small arterioles but not in hepatocytes in which LDLR is widely distributed [36]. In contrast, LRP8 (ApoER2), which has 50 percent homology with VLDLR, is mainly expressed in brain, testis, and placenta [37]. VLDLR primarily modulates the extra-hepatic metabolism of TG-rich lipoprotein. The structure of VLDLR is highly homologous to that of LDLR (Figure 1). VLDLR contains eight cysteine-rich LDLR type A repeat domains compared to seven in LDLR. ApoE-containing VLDL not only binds to VLDLR, but to LDLR, LRP8, LRP1, LRP2, and likely LRP6 as well. Unlike LDLR, VLDLR is not linked to a feedback mechanism for its expression in response to intracellular VLDL [38]. VLDLR expression is directly regulated by PPAR-γ. Accordingly, pioglitazone (agonist of PPAR-γ) increases Vldlr mRNA expression and protein level in 3T3-L1 preadipocytes (mouse fibroblasts). Mice treated with pioglitazone exhibit enhanced conversion of plasma TG to epididymal fat. This response is absent in VLDLR deficient mice, which implies that VLDLR plays a key role in fat deposition [39].

VLDLR has an important function in postprandial chylomicron and VLDL clearance by up-regulating lipoprotein lipase (LPL)-mediated TG hydrolysis and direct uptake of TG-rich lipoproteins in endothelial cell [3]. VLDL and LPL have similar distribution patterns in peripheral tissues. Vldlr−/− mice have diminished LPL expression, which causes increased plasma TG; Vldlr−/− mice exhibit 250 percent higher TG levels and 60 percent lower chylomicron uptake compared to those in wildtype [3]. Therefore, VLDLR actively functions as a lipoprotein receptor by uptake of TG-rich VLDL (not LDL) and provides sufficient energy substrates in peripheral tissues. Vldlr−/− mice also exhibit low adipose tissue mass without significant change in their plasma lipoprotein profiles. This latter finding is due to the fact that HDL has the greatest contribution to lipoproteins in mice [11]. Conversely, adenovirus transfer of Vldlr cDNA to the liver increases cholesterol clearance by binding apoE-containing lipoprotein in mice model [40,41]. Expression of VLDLR increases with fasting, which also increases fatty acid binding protein and acetyl CoA synthase to provide a sufficient energy source for vital organs such as the heart and brain [42]. The exact molecular mechanisms of VLDLR function in lipoprotein metabolism are not fully understood.

**LOW-DENSITY LIPOPROTEIN-RELATED PROTEIN 6 (LRP6)**

LRP6 is a member of the LDLR family, which together with LRP5 has the unique structure and function as an essential co-receptor for Wnt/β-catenin signaling [43]. In particular, extracellular domain of LRP6 has three types of subdomains, including LDLR type A repeats, EGF-like domain, and YWTD-type β-propeller domain (Figure 1). LRP6 is comprised of three large clusters of ligand binding repeats, each with ligand binding functions with Wnt, DKK1, and lipoprotein particles, whereas LDLR and VLDLR contain only a single ligand-binding repeat cluster. Upon binding of Wnt to cell surface receptor Frizzled and its co-receptors LRP5/6 complex, the cytoplasmic tail of LRP6 is phosphorylated by CK1 and glycogen synthase kinase 3β (GSK-3β), leading to Dvl recruitment and binding of Axin complex. This results in stabilization of β-catenin and its translocation onto the nucleus, where it binds
to transcription factors such as T cell factor (TCF)/lymphoid enhancer factor (LEF) and stimulates various gene expressions [44].

A missense mutation in \textit{LRP6} at a highly conserved residue of an EGF domain has been linked to autosomal-dominant early onset of cardiovascular disease and metabolic syndrome traits, including hyperlipidemia, diabetes, osteoporosis, and hypertension [2]. Patients who carry the \textit{LRP6}\textsubscript{R611C} mutation exhibit elevated serum LDL cholesterol, TG, and fasting glucose level, which together constitute the metabolic syndrome, a major risk factor for atherosclerosis and myocardial infarction. These associations strongly suggest a pluripotent effect of this gene.

Common genetic variations within \textit{LRP6} also have been associated with plasma LDL levels, implicating \textit{LRP6} as a potential regulator of lipid metabolism and a novel target for pharmacological interventions [45]. Liu et al. [46] have demonstrated that an intact \textit{LRP6} function is essential for normal LDL clearance, whereas the \textit{LRP6}\textsubscript{R611C} mutation results in reduced \textit{LRP6} membrane expression and impaired LDL clearance. Splenic macrophages of \textit{LRP6}\textsuperscript{-/-} mice display reduced LDL uptake compared to wild-type mice. Peripheral B-lymphocytes of \textit{LRP6}\textsubscript{R611C} mutation carriers exhibit significantly impaired LDL internalization despite similar affinities for apoB at neutral pH. \textit{LRP6} augments cellular LDL binding and uptake, both in LDLR dependent and independent manner. According to Ye et al. [9], \textit{LRP6} co-localizes with LDLR and regulates its clathrin-dependent internalization (Figure 2). \textit{LRP6} forms a complex with clathrin and ARH and is required for LDL clearance via clathrin-mediated internalization. Human fibroblast of \textit{LRP6}\textsubscript{R611C} mutant carriers exhibit impaired LDL internalization despite similar affinities for apoB at neutral pH. Preliminary work in our laboratory has shown that \textit{LRP6} not only regulates LDL and TG clearance, but it is also involved in synthesis of TG and fatty acids.

Keramati et al. have reported increased expression and co-localization of \textit{LRP6} with PDGF receptor \(\beta\) in human atherosclerotic coronary arteries [47]. Wild-type \textit{LRP6} forms a complex with platelet-derived growth factor receptor (PDGFR)-\(\beta\) and triggers its lysosomal degradation. This effect reduces vascular smooth muscle cell proliferation and is considered protective against atherosclerosis. Conversely, the \textit{LRP6}\textsubscript{R611C} mutation significantly activates PDGF signaling and increases PDGF-dependent cell-cycle activity in smooth muscle cell. This causes increased smooth muscle proliferation, which is an important component of atherosclerosis development.

**LOW-DENSITY LIPOPROTEIN-RELATED PROTEIN 1**

\textit{LRP1} (also known as \(a2\)-macroglobulin receptor or CD91) is ubiquitously expressed in most tissues and abundantly exists in the liver and brain. \textit{LRP1} is composed of a large ligand binding subunit (515-kDa), which recognizes more than 40 ligands, and a relatively small transmembrane fragment (85-kDa) (Figure 1). \textit{LRP1} harbors ligands as a cargo transporter and modulates physiologic processes including inflammatory response in the lung [48], amyloid \(\beta\) uptake in blood-brain barrier [49], and clearance of factor VIII in the blood coagulation process [50]. A number of proteases and protease inhibitor complexes are regulated by \textit{LRP1}-mediated pathway [51]. \textit{LRP1} also functions as a receptor for removal of apoE-rich chylomicron remnant; i.e., it shuttles dietary lipid from intestine to liver. \textit{LRP1} is likewise involved in the clearance of apoE-rich lipoprotein, including VLDL in liver, smooth muscle cells, and macrophages.

\textit{LRP1} is essential for the maintenance of vascular integrity during embryonic development. In adult life, \textit{LRP1} plays an essential role in protecting against atherosclerosis by reducing vascular smooth muscle cell (VSMC) proliferation and regulating levels of PDGF receptor in the vessel wall [52]. \textit{Lrp1}\textsuperscript{-/-} mice increase susceptibility to development of atherosclerotic lesions by greater secretion of apoE, reduced uptake of remnant in macrophages, and reduced HDL plasma level [4]. \textit{LRP1} inactivation likewise increases postprandial dyslipidemia and atherosclerosis in \textit{Lrp1}\textsuperscript{-/-} mice [53]. In addition to
cholesterol homeostasis, LRP1 is involved in fatty acid uptake. Lrp1−/− fibroblasts decrease the uptake of fatty acid, resulting in an increase of free fatty acid and redistribution to the liver [12]. Statin intervention in rats has shown to up-regulate both LRP1 and LDLR via activated SREBP-2 related pathway, resulting in protection against atherosclerosis [54].

LOW-DENSITY LIPOPROTEIN-RELATED PROTEIN 2, MEGALIN

LRP2, also named megalin for its huge molecular structure, is a member of the LDLR family that is abundantly expressed in different epithelial cell types [55]. LRP2 is an endocytic receptor for diverse ligands, including lipoprotein, steroid, and retinoid. Renal re-absorption of a variety of molecules, in particular vitamin B₁₂ and HDL, is an important function of LRP2, which is positively regulated by PPAR-α/β [13]. LRP2 is composed of extracellular ligand binding domain, transmembrane fragment, and cytoplasmic tail containing three NPxY motifs (a total molecular weight of 517 kDa) (Figure 1). Similar to LRP1, receptor function of LRP2 is connected to proteolysis between the transmembrane anchor and cytoplasmic motif. Ligand binding to LRP2 triggers shedding of its ectodomain by protein kinase C (PKC)-induced matrix metalloproteinase. Cytoplasmic C-terminal fragment is cleaved by β-secretase, resulting in its release into the cytoplasm, whereby it presumably functions as a transcriptional regulator in the nucleus [56]. Similar to LRP5/6, LRP2 needs mesoderm development (MESD) chaperone, which prevents misfolding of LRP2 [57]. LRP2 is involved in embryonic renal development, including vitamin D homeostasis, sex hormone signaling, and holoprosencephaly [58-60]. Genetic variations in LRP2 have been associated with elevated total cholesterol and LDL in patients with dyslipidemia [61]. LRP2 harbors cubilin, which bind to HDL, thus providing an important role in endocytosis of HDL cholesterol [5]. The role of LRP2 in dyslipidemia and atherosclerosis is far from complete.

LOW-DENSITY LIPOPROTEIN-RELATED PROTEIN 5

LRP5 structure is analogous to that of the LRP6 and similarly functions as a co-receptor for Wnt/β-catenin signaling. LRP5 is well known for its affect on bone mass density and mineralization. Loss of function mutations are associated with osteoporosis pseudoglioma [62], and gain of function mutations are associated with high bone density [63]. LRP5 may be involved in mineralization of the atherosclerotic lesion [64]. Apoe−/− mice fed with western diet (42 percent kcal from fat and 0.2 percent cholesterol) show increased LRP5 expression. Interestingly, Apoe−/− mice on a Western diet develop calcified plaque, while Lrp5−/− or Apoe−/−, Lrp5−/− double knockouts have no or minimal calcified lesions [65]. Accordingly, Ldlr−/− mice fed either chow (18 percent kcal from fat) or Western diet have high expression levels of Lrp5 in aortic tissue [66]. Lrp5−/− mice exhibit decreased hepatic clearance of chylomicron remnants, plasma cholesterol levels, and impaired insulin secretion [67]. Further investigation is necessary to clarify the role of LRP5 in calcification of atheromatous plaque and lipoprotein endocytosis.

CONCLUSIONS

Members of the LDLR family of proteins dynamically bind and internalize apoE- and apoB-containing lipoprotein, including LDL, VLDL, and chylomicron remnant. Defect in these receptors is associated with dyslipidemia and atherosclerosis in mammals. LDL defect is the most common cause of familial hypercholesterolemia, which is associated with very high plasma LDL levels, accounted for by impaired LDL clearance and enhanced cholesterol biosynthesis. VLDLR modulates the binding and uptake of apoE-containing lipoprotein, including chylomicron and VLDL, and regulates TG level in plasma. LRP6, well known as co-receptor for Wnt signal pathway, is a unique member of this receptor family. Recent work in our laboratory and by others has shown that LRP6 also functions as a regulator of cholesterol and lipid homeostasis, glucose metabolism, and blood pressure. Preliminary work in our
laboratory has shown that this receptor not only regulates LDL and TG uptake, but it is also involved in synthesis of TG and fatty acids. In addition, LRP6 plays a major role in vascular integrity and protection against atherosclerosis, in part by its anti-proliferative effects. Therefore, LDLR family members are principal targets of novel therapeutics for metabolic risk factors and diseases. While current lipid lowering drugs such as statins have been very effective in reducing serum LDL levels in the general population, there remain a significant number of patients with extremely high LDL levels that show no or modest responses. Furthermore, these drugs have no or small effects on serum TG and HDL. Inflammatory processes and vascular smooth muscle cell proliferation also play a pivotal role in cardiovascular diseases. Therefore, other members of the LDLR family may be targets of novel therapeutic for the most common form of hyperlipidemia known as combined familial hyperlipidemia. For instance, targeting LRP6 may reverse diverse pathophysiological processes in atherosclerotic diseases, including increased TG/VLDL synthesis, elevated lipolysis, decreased LDL clearance, and enhanced VSMC proliferation.

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