Much of our understanding of normal low-density lipoprotein (LDL) cholesterol metabolism can be traced to studies in human carriers of naturally occurring mutations, which perturb proteins acting at key metabolic checkpoints. The best known among these single-gene disorders is familial hypercholesterolemia (FH), which in the heterozygous state affects ≈1 in 250 to 500 individuals and classically results from a loss-of-function mutation in 1 allele of the LDLR gene encoding the LDL receptor, primarily in the liver. Untreated FH heterozygotes have plasma LDL cholesterol levels 2x to 3x normal and begin to express atherosclerosis symptoms and signs in the third to fourth decade of life. FH heterozygotes respond well and begin to express atherosclerosis symptoms and signs in the third to fourth decade of life.2 FH heterozygotes respond well to multidimensional treatment, including lifestyle modification plus medications, primarily statins plus cholesterol absorption inhibitors, and bile acid sequestrants, which all act to upregulate the 1 functional (wild-type) LDLR allele.3 In contrast, the much rarer homozygous form of FH (HoFH) affects 1 in 250000 to 1000 000 individuals, classically results from loss-of-function mutations in both LDLR alleles, is associated with plasma LDL cholesterol levels 4x to 10x normal, is expressed clinically by the second decade with significant lipid deposits affecting skin, tendons, and arteries, and is characterized by minimal response to traditional lipid-lowering therapies.4 In fact, the minuscule biochemical response to statin treatment in patients with HoFH confirms that statins reduce plasma LDL cholesterol by upregulating expression of LDL receptors, which are functionally absent in these patients.5

Care Gap in HoFH: Potential Role for Gene Therapy

The standard of care for HoFH is weekly or biweekly plasmapheresis or LDL apheresis.5 However, this treatment is not universally available, and although it has likely improved longevity, patients with HoFH still develop early cardiovascular disease, including premature coronary atherosclerosis and significant left ventricular outflow tract disease.6 Liver transplantation has also been attempted to control LDL cholesterol levels, but it requires lifelong immunosuppression. Newer drug therapies recently approved for HoFH, namely lomitapide and mipomersen, target the production of apolipoprotein B–containing lipoproteins, but their adverse effects include hepatosteatosis, which may have severe long-term consequences.9

Gene therapy using safe vectors to deliver functioning LDL receptors offers the possibility of stable, long-term correction of the fundamental defect in HoFH.10 A pilot study in 1995 reported rather invasive liver-directed gene therapy using recombinant retroviruses in 5 FH homozygotes, with inconsistent and short-lived efficacy.11 The need for substantial technological improvements have since that time been recognized as essential before gene therapy could be ready for prime time in the treatment of HoFH. By analogy with the incremental improvements made to flying machines in the early days of aviation, progress in human gene therapy has been characterized by stepwise evolutionary advances rather than swift revolutionary changes. Refinements include development of vectors that can be delivered intravenously and can efficiently transduce liver cells with stable long-term LDL receptor expression. For instance, vectors based on serotype 8 of the adenovasociated virus (AAV8) seem to hold promise as a delivery system.12 A complementary approach to improve efficacy further involves inserting a modified version of the LDL receptor that has been engineered to enhance its ability to catabolize circulating LDL particles. This strategy is analogous to the use of alipogene tiparvovec (AAV1-LPLS447X; Glybera), a type of gene therapy for lipoprotein lipase deficiency in which a naturally occurring hyperfunctional form of the enzyme was inserted into an adenoviral vector.13 But although there is a dearth of naturally occurring human gain-of-function mutations in the LDLR gene, there are clever approaches that might enhance and prolong LDL receptor function based on current awareness of its intracellular itinerary. Therefore, it has been hypothesized that LDLR gene therapy for HoFH could be significantly enhanced by increasing functional plasma membrane receptor density by either enhancing LDLR gene expression or preventing receptor degradation.

The Liver Giveth, the Liver Taketh Away

LDLR expression is regulated by the transcription factor sterol regulatory element binding protein–2 (SREBP-2).14 Low cellular cholesterol activates SREBP-2–mediated LDLR transcription. Inhibition of hepatic 3-hydroxy-3-methyl–glutaryl-coenzyme A reductase by statins decreases cellular cholesterol thereby activating SREBP-2, and increasing LDLR expression leading to decreased plasma LDL cholesterol.14 LDL receptors are recycled several times until they are finally degraded by 1 of 2 recently discovered pathways (Figure). More than a decade ago, proprotein convertase subtilisin/kexin type 9 (PCSK9) was discovered to promote the degradation of LDL receptors by cleaving the LDL receptor at a specific site and targeting it for degradation within minutes, thus reflecting a possible target for the treatment of FH.15

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

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of the LDL receptor. Expression of both PCSK9 and the LDLR is regulated by the transcription factor SREBP-2: low cellular cholesterol activates expression of SREBP-2, which in turn increases expression of both LDLR and PCSK9. Autocatalytic processing of PCSK9 in the endoplasmic reticulum releases its prodomain, which remains associated with PCSK9, thereby blocking its catalytic site. The active protein is secreted and its prodomain, which remains associated with PCSK9, thereby processing of PCSK9 in the endoplasmic reticulum releases the prodomain, which remains bound to PCSK9. Secreted active PCSK9 binds the extracellular epidermal growth factor-A domain of the LDLR at the plasma membrane. LDLR/PCSK9 is internalized by clathrin-dependent endocytosis and定向 toward lysosomal degradation. These degradation mechanisms explain why human genetic gain-of-function mutations in PCSK9 raise LDL cholesterol levels, whereas loss-of-function mutations lower LDL cholesterol levels and reduce cardiovascular disease risk. A new class of cholesterol-lowering drugs is based on blocking the function of PCSK9.

Recently, Zelcer et al. discovered the inducible degrader of the low-density lipoprotein receptor (IDOL). The gene expressing IDOL is also known as MYLIP. Functioning as an E3 ubiquitin ligase, IDOL mediates ubiquitination and degradation of the LDL receptor. IDOL is a liver X receptor-target gene: increased cellular cholesterol activates liver X receptor and increases IDOL expression. IDOL functions as a homodimer, and together with the E2-ubiquitin conjugating enzyme UBE2D, IDOL binds to the intracellular domain of the LDLR at the plasma membrane and promotes the attachment of ubiquitin chains (Ub). Internalized ubiquitinated LDLR within endosomes is delivered to multivesicular bodies (MVB) via the protein endosomal sorting complexes required for transport (ESCRT), becomes deubiquitinated, and is targeted for lysosomal degradation. An intracellular pathway of IDOL-mediated LDLR degradation has also been described. Introduced mutations (K809R/C818A) in the LDLR cytoplasmic domain (*) blocks binding of IDOL, ultimately preventing LDLR lysosomal degradation. This increases the abundance of active LDLR at the plasma membrane, enhances LDL binding and uptake, thereby lowering plasma LDL concentrations.

Figure. Sterol regulatory element binding protein-2 (SREBP2)–proprotein convertase subtilisin/kexin type 9 (PCSK9) and liver X receptor (LXR)-inducible degrader of the low-density lipoprotein receptor (IDOL) pathways control abundance of the LDL receptor in the liver. Right, PCSK9 pathway. 1. Activation of the SREBP2 by low cellular cholesterol increases mRNA expression of both the low-density lipoprotein receptor (LDLR) and PCSK9. 2. Autocatalytic processing of PCSK9 in the endoplasmic reticulum (ER) releases the prodomain, which remains bound to PCSK9. 3. Secreted active PCSK9 binds the extracellular epidermal growth factor-A domain of the LDLR at the plasma membrane. 4. LDLR/PCSK9 is internalized by clathrin-dependent endocytosis. 5. Binding of PCSK9 to the LDLR directs the receptor toward lysosomal degradation and (6) prevents LDLR recycling from endosomes back to the plasma membrane. 7. An intracellular pathway also exists, in which active PCSK9 binds the LDLR in the Golgi apparatus and targets LDLR for lysosomal degradation. 8. An introduced mutation (L318D) in the LDLR epidermal growth factor domain (x) blocks binding of PCSK9, preventing lysosomal degradation. 9. This increases the abundance of active LDLR at the plasma membrane, enhances LDL binding and uptake, thereby lowering plasma LDL concentrations. Left, IDOL pathway. 10. Increased cellular sterol leads to transcriptional activation of the liver X receptor (LXR) pathway and increased expression of the IDOL. 11. As a homodimer, and in cooperation with E2-ubiquitin conjugating enzyme (UBE2D), IDOL binds to the intracellular domain of the LDLR at the plasma membrane and promotes the attachment of ubiquitin chains (Ub). 12. Internalized ubiquitinated LDLR within endosomes is delivered to multivesicular bodies (MVB) via the protein endosomal sorting complexes required for transport (ESCRT1), becomes deubiquitinated, and is targeted for lysosomal degradation. 13. IDOL leads to recognition by endosomal sorting complexes required for transport, deubiquitination, and subsequent multivesicular body–dependent lysosomal degradation.
on LDL receptor present in the plasma membrane,29,30,32 but an intracellular pathway of receptor ubiquitination has also been described.27,32 Ultimately, IDOL functions to decrease the number of functional LDL receptors at the cell surface.27 Consistent with this mechanism, genome-wide association studies have shown that common variations at the MYLIP/IDOL locus were associated with variation in LDL cholesterol in humans.33–35 Furthermore, the naturally occurring gain-of-function IDOL p.N342S variant has been associated with elevated plasma cholesterol.36

**Better Vector, Better Insert**

In the current issue of Circulation Research, Somanathan et al37 describe the development and efficacy of AAV8 vector expressing gain-of-function human (h) LDLR variants. By exploiting the understanding of LDL receptor degradation, mutations were introduced into LDLR cDNA that would alter amino acids known to be required for hPCSK9- and hIDOL-mediated degradation; the mutations were predicted to render the receptor resistant to the degradation mechanisms described above. When expressed in the liver, the net effect of these resistant mutant receptors would be an increase in cell surface density of LDL receptors, ultimately amplifying the reduction in plasma LDL cholesterol (Figure). Initial screening in HEK293 cells led to the selection of hLDLR-L318D and LDLR-K809R/C818A variants, both of which functioned as well as wild-type receptors, but escaped degradation when cells were co-expressed with hPCSK9 or hIDOL, respectively. In a mouse model of human HoFH, vectors expressing wild-type hLDLR or the variant hLDLR-L318D were co-injected with hPCSK9. When compared with wild-type hLDLR, hLDLR-L318D protein expression persisted in liver, and non-high-density lipoprotein (HDL) cholesterol was decreased by a further 15%. In similar experiments, compared with wild-type hLDLR, hLDLR-K809R/C818A co-injected with hIDOL led to a further 20% reduction in non-HDL cholesterol. Protection from IDOL-mediated degradation was observed only when low levels of the hLDLR-K809R/C818A receptor construct were injected. An AAV8.LDLR-L318D/K809R/C818A vector that harbored all 3 amino acid substitutions conferred partial resistance to degradation in mice co-injected with either hPCSK9 or hIDOL. Thus, these amino acid substitutions in the human LDL receptor confer partial resistance to PCSK9- and IDOL-mediated degradation, with improved lowering of non-HDL cholesterol levels in mice. These novel and exciting findings reveal a new potential strategy for improvement in gene therapy for the treatment of patients with HoFH. The experiments provide an important proof of concept and clearly show that the strategy works in cell culture and in mice. Nevertheless, some outstanding questions remain to be resolved.

**A Time for Questions**

The experiments of Somanathan et al37 show that targeted mutations can help the LDL receptor to escape degradation, but only when PCSK9, IDOL, or both are also overexpressed in parallel within AAV8 vectors. It remains to be determined if such modified receptors can escape degradation from endogenous PCSK9 and IDOL at physiological levels, such as those present in the livers of patients with HoFH. The effect of LDLR mutations that protected from IDOL-mediated degradation on non-HDL cholesterol reductions in mice was more modest and was only effective at low levels of LDL receptor vector expression. As pointed out by the authors, this could be because of the presence of other amino acids in the LDL receptor that facilitate binding and degradation by IDOL. However, it could also be because of the known reduced effect of IDOL on hepatic LDL receptors in the liver.27 The latter hypothesis will require testing in humans to determine how these competing mechanisms are integrated to produce a net effect. In the model systems studied, the resistance of the LDL receptor from PCSK9- or IDOL-mediated degradation resulted in further decreases in plasma non-HDL cholesterol levels of 12% to 20%. However, patients with HoFH require large decreases in LDL cholesterol to affect disease progression, and thus it is not clear whether the effect of these vectors expressing gain-of-function LDL receptors will be clinically significant.

Also, inhibition of expression of PCSK9 by several new investigational agents35,36 is being explored as another type of adjunctive treatment that could be useful in patients with HoFH. However, the efficacy of such treatments would depend on having functional PCSK9 expressed that can interact with the transfected mutant LDL receptor protein. If hLDLR-L318D is delivered and expressed in the liver, the effect of concomitant PCSK9 inhibition would be predicted as neutral because the mutation disturbs the LDL receptor–PCSK9 interaction. If PCSK9 cannot interact with the mutant LDL receptor, then knocking down endogenous PCSK9 would not be expected to have any incremental effect. Again, in vivo studies would be important to demonstrate integrated effects of these theoretical mechanisms.

**One Small Step for Gene Therapy, One Giant Leap for HoFH?**

Given the recent disappointments in the HDL field, there is intensified interest in new approaches for reducing LDL cholesterol. Translational investigators have refocused their attention on both heterozygous FH and HoFH as human model systems of extreme LDL cholesterol elevations. Although pharmacological approaches—such as lomitapide and mipomersen—have monopolized attention recently, the work of Somanathan et al37 reminds us of the slow and steady progress being made in gene therapy for HoFH. Just as tinkering with mechanical aspects of early flying machines ultimately led to the success that formed the basis of modern aviation, incremental intelligent advances in early flying machines ultimately led to the success that formed the basis of modern aviation, incremental intelligent advances in gene therapy may one day help patients with HoFH attain the sweet dream of stable, safe, effective, and definitive therapy for this serious condition.

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Gene Therapy for Hypercholesterolemia: Sweet Dreams and Flying Machines
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