Novel mechanisms regulating LDL receptor activity

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Hypercholesterolemia is a causal and treatable risk factor of atherosclerotic cardiovascular diseases (ASCVD)[1]. The most important determinant of LDL-cholesterol (LDL-C) levels in plasma is the hepatic removal of circulating LDL by binding to LDL receptors (LDLR) for subsequent endocytosis and degradation[2]. The expression of LDLR is tightly regulated by transcription factors, proteasomal and lysosomal degradation, endosomal recycling, and cleavage at the cell surface[1,2]. The unravelling of this complex regulation led to the development of drugs that effectively lower plasma levels of cholesterol and, as the consequence, risk of ASCVD[1]. Nevertheless, there is still a considerable gap of knowledge in the understanding of the LDLR pathway. To identify novel regulators of LDL uptake into the liver, we performed an image-based genome-wide RNA interference (RNAi) screen in Huh-7 human hepatocarcinoma cells. At an RSA p-value cutoff of $p < 10^{-3}$, interference with 54 and 37 genes decreased and increased LDL uptake, respectively. Gene Ontology (GO) enrichment analysis showed significant clustering for genes whose loss of function decreased LDL uptake. Functional clustering of these genes with the STRING tool revealed four major groups: the ribosome, the proteasome, the spliceosome, and vesicular transport. By targeted experiments we confirmed the limiting role of two multiprotein complexes involved in RNA splicing or vesicular transport and unravelled the underlying mechanisms. We also collected genetic data in humans that support the limiting role of these two gene clusters / multiprotein

complexes for the regulation of LDL receptor activity and the determination of LDL-cholesterol levels.

1 Ference BA, Ginsberg HN, Graham I, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement fromthe European Atherosclerosis Society Consensus Panel. *European Heart Journal*. 2017;38:2459–2472.

2. Zanoni P, Velagapudi S, Yalcinkaya M, Rohrer L, von Eckardstein A. Endocytosis of lipoproteins. *Atherosclerosis*. 2018;275:273–295.