CRISPR-based Gene Editing Enhances LDLR Expression and Boosts LDL-C Uptake in Familial Hypercholesterolemia

Marina Cherniavsky Lev, Lior Gefen, Ygal Levy, Ira Gotliv, Lior Izhar, Rafi Emmanuel

Emendo Biotherapeutics

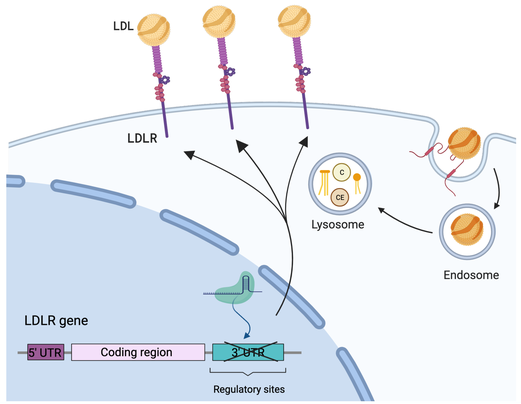
emendo bio
NEXT GENERATION CRISPR
MAKING ANY GENE TARGETABLE

INTRODUCTION

Familial hypercholesterolemia (FH) is a prevalent autosomal dominant disorder characterized by a lifelong elevation of low-density lipoprotein cholesterol (LDL-C), which results in early-onset atherosclerosis and coronary events. About 85% to 90% of genetically confirmed FH is caused by pathogenic mutations in the LDLR gene, haploinsufficiency of which leads to reduced LDL-C uptake[1,2]. Lifelong lipid-lowering medications, such as Statins and Ezetimibe, are currently available, however, they are often intolerable by some of the patients and fail to attain desired LDL-C levels[3]. A more recent therapeutic approach is based on subcutaneously injected monoclonal antibodies that transiently inhibit proprotein convertase subtilisin/kexin type 9 (PCSK9), a protein that promotes LDLR lysosomal degradation[4,5]. Such approach, however, is frequently insufficient as a monotherapy, and is thus prescribed in combination with a Statin therapy[6]. Here we present a novel, direct and long-lasting therapeutic strategy for upregulation of LDLR expression by truncating a section of the LDLR 3' UTR, which contains sites that negatively regulate LDLR expression, via CRISPR-based gene editing. Editing strategy was tested in HepG2 cell line, FH patient-derived Lymphoblastoid cell lines (LCLs) and mouse hepatoma cell line Hepa1-6. Excision of the 3'UTR was confirmed by ddPCR and LDLR mRNA levels were quantified by qRT-PCR. Total and surface LDLR levels were determined by Western Blot and flow cytometry, respectively, using specific antibodies. Finally, the effect of 3'UTR excision on cholesterol uptake was assessed by measuring the cellular intake of fluorescently labeled LDL-C, using flow cytometry. Excision efficiency in HepG2 cells was about 50%. Excised cells showed a 2-fold upregulation of LDLR mRNA levels and a 6-fold increase in surface LDLR as compared to non-treated cells. 3'UTR excision resulted in a 3-fold increase in the uptake of LDL-C. Patient-derived LCLs showed similar outcomes, with a 4-fold increase in LDL-C uptake. In addition, a comparative analysis showed that our strategy outperforms PCSK9 knockout (KO) and Statins in increasing LDL-C uptake. These findings support our CRISPR-based gene editing strategy of truncating regions responsible for rapid LDLR mRNA turnover to enhance its expression and boost LDL-C uptake. This unique approach could prove useful for a variety of hypercholesterolemia-related disorders.

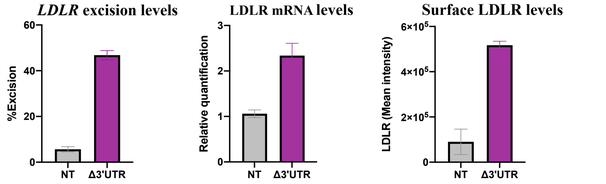
1. Jiang, L., *et al.* (2015). Sci. Rep.
2. Soutar, A.K., *et al.* (2007). Nat. Clin. Pract. Cardiovasc. Med.
3. Harada-Shiba, M., *et al.* (2018). J. Atheroscler. Thromb.
4. Ito, M.K., *et al.* (2017). J. Clin. Pharmacol.
5. Chan, J.C.Y., *et al.* (2009). Proc. Natl. Acad. Sci.
6. Lui, D.T.W., *et al.* (2021). J. Endocr. Soc.

THERAPEUTIC STRATEGY



RESULTS

3'UTR excision of *LDLR* leads to its upregulation in HepG2 liver human cell line

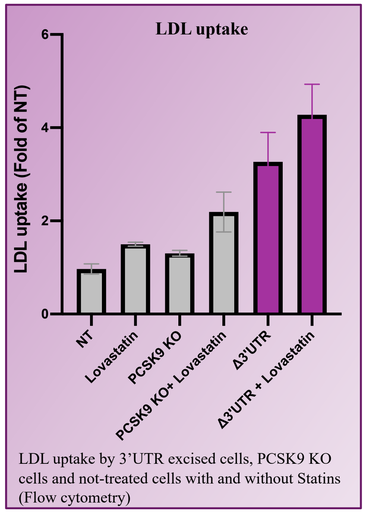


Excision percentages of 3'UTR *LDLR* (ddPCR)

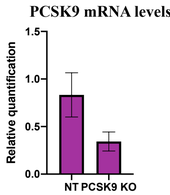
LDLR mRNA expression levels in excised and not-treated cells (qRT-PCR)

Surface LDLR expression in excised and not-treated cells (Flow cytometry)

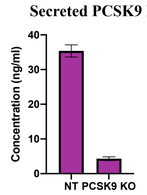
3'UTR excision of *LDLR* is superior to PCSK9 depletion and Statins treatment



LDL uptake by 3'UTR excised cells, PCSK9 KO cells and not-treated cells with and without Statins (Flow cytometry)

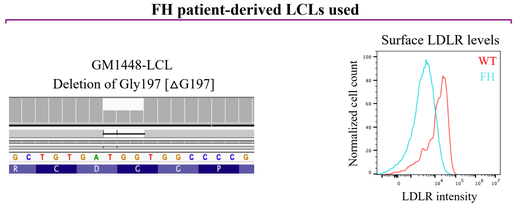


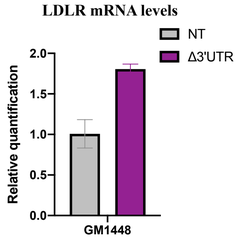
PCSK9 mRNA expression levels following CRISPR-based PCSK9 KO (qRT-PCR)



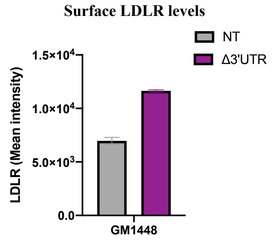
Secreted PCSK9 levels following CRISPR-based PCSK9 KO (ELISA)

3'UTR excision of *LDLR* rescues impaired LDL uptake in FH patient-derived Lymphoblastoid cell lines (LCLs)

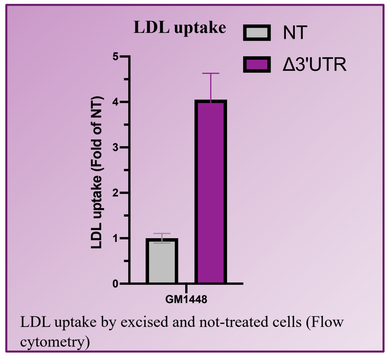




LDLR mRNA expression levels in excised and not-treated cells (qRT-PCR)

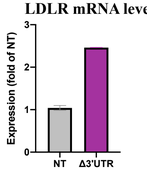


Surface LDLR expression in excised and not-treated cells (Flow cytometry)

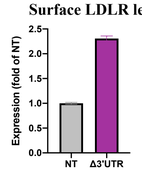


LDL uptake by excised and not-treated cells (Flow cytometry)

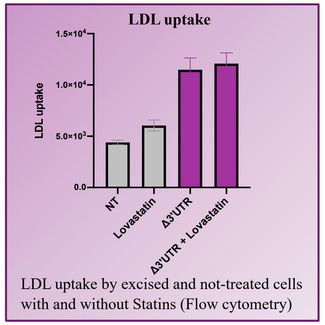
Relevance of a mouse model for efficacy studies



LDLR mRNA expression levels in excised and not-treated cells (qRT-PCR)

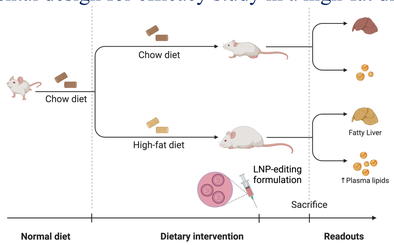


Surface LDLR expression in excised and not-treated cells (Flow cytometry)



LDL uptake by excised and not-treated cells with and without Statins (Flow cytometry)

Experimental design for efficacy study in a high-fat diet mouse model



CONCLUSIONS

- **Truncating a section of the *LDLR* 3' UTR via CRISPR-based gene editing resulted in an upregulation of LDLR expression and increased LDL uptake.**
- **This novel, direct and long-lasting therapeutic strategy was found to be superior to PCSK9 depletion and Statins in cell models.**
- **This unique approach could prove useful for a variety of hypercholesterolemia-related disorders.**