Session 11a - Concurrent Session II A: Apolipoproteins, Lipoproteins and Lipid Metabolism

117 - Transgelin: A New Gene Involved in LDL Endocytosis in Liver Cells Identified by a Whole-genome Crispr-cas9 Screen

May 14, 2019, 5:45 PM - 6:00 PM | Grand Ballroom Salon E

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Disclosures

D. Lucero: None. **M. Mendelson:** None. **P. Islam:** None. **L.A. Freeman:** None. **E.B. Neufeld:** None. **J. Tang:** None. **C. Combs:** None. **Y. Li:** None. **A.T. Remaley:** None.

Abstract

Familial hypercholesterolemia is caused by mutations in either LDL receptor (***LDLR***), ApoB-100, ***LDLRAP1***, or ***PCSK9***, but ~30% of patients lack these mutations, suggesting that unknown genes are also involved in LDL uptake.**Aim:**To identify novel genes involved in hepatic LDL uptake by using a whole-genome scale CRIPSR-Cas9 screen. **Methods:**Stable Cas9-expressing HepG2 cells were transfected with lentivirus (MOI: ~0.4) containing a human whole-genome CRISPR Cas9 knock-out (KO) library (Brunello, Addgene) that included 76,441 guide RNAs (gRNA) targeting 19,114 genes (4 gRNA/gene). Transfected cells were incubated 4h with AlexaFluor568-LDL (50 µg/ml). Cells with the 6% lowest LDL uptake were sorted by FACS and gRNAs were PCR-amplified and sequenced by Next-Generation Sequencing. Enrichment of gRNAs compared to the original library was determined with MAGeCK software. Genes were ranked by average of three most enriched gRNAs. **Results:** *LDLR was*the second most enriched gene, validating the screen.The 15 most enriched genes were selected for further testing by examining LDL uptake after stably knocking out each gene in HepG2 cells. Besides ***LDLR,*** which showed an 80% reduction in uptake, the following genes also showed decreased LDL uptake: ***C4orf33*** (-22%),***SYRNG*** (-33%) and ***TAGLN*** (-27%) ($p<0.01$). The relation between ***TAGLN***, and actin bundling protein, and LDL was evidenced in human populations. GWAS showed that a common variant,rs641620, intronic to ***TAGLN*** (transgelin) is associated with LDL-cholesterol ($p=8.80E^{-7}$) and triglycerides ($p=1.80E^{-30}$). ***TAGLN***-KO cells also showed reduced uptake of VLDL (-24%), transferrin (-39%) and lucifer yellow (-28%) ($p<0.05$). Interestingly, ***TAGLN***-KO cells showed increased ***LDLR*** mRNA levels and higher ***HMGCR*** and ***MVK*** mRNA levels than control cells ($p<0.01$). Confocal microscopy showed that ***TAGLN***-KO cells are defective in LDLR internalization.**Conclusion:** ***TAGLN*** plays a central role in endocytosis, including LDL-LDLR internalization. ***TAGLN*** loss triggers compensatory ***LDLR*** transcription and increases cellular cholesterol synthesis. Based on its effect on VLDL uptake and its association with both increased LDL-cholesterol and high triglycerides, ***TAGLN*** could be a candidate gene for Familial Combined Hyperlipidemia.