SUPPLEMENTAL MATERIAL

Detailed Methods

Screening of guide RNAs. Candidate guide RNAs were designed by visual inspection of the sequences of exon 1 of the human *PCSK9* gene. Guide RNAs were screened for on-target activity in HEK 293T cells as previously described.[1] The guide RNA used (protospacer 5'-GGTGCTAGCCTTGCGTTCCG-3') was chosen because of its combination of high on-target activity *in vitro* and favorable off-target profiling (see Supplemental Figures I and II). An irrelevant guide RNA was used as a control (protospacer 5'- GTGCTTGATTGAGCAACCTC-3').

Generation of adenoviruses. The *Streptococcus pyogenes* CRISPR-Cas9 system and the guide RNA protospacers were inserted into the Adeno-X vector (Clontech) as previously described.[1] The Penn Vector Core at the University of Pennsylvania used these vectors to generate recombinant adenoviral particles (designated CRISPR-*PCSK9* and CRISPR-control).

Animal studies. All procedures used in animal studies were approved by the pertinent Institutional Animal Care and Use Committees at Harvard University and were consistent with local, state, and federal regulations as applicable. *Fah*$^{-/-}$*Rag2*$^{-/-}$*Il2rg*$^{-/-}$ (FRG KO) breeder mice on the C57BL/6 background were obtained from Yecuris Corporation. Mice were maintained on NTBC (also called Nitisinone; Yecuris) prior to transplantation according to the manufacturer's instructions. Twenty-four hours prior to transplantation, mice that were one to three months of age underwent intraperitoneal injection with 1×10^9 pfu of adenovirus expressing the secreted form of urokinase-type plasminogen activator (Yecuris). For transplantation, 1×10^6 primary hepatocytes (HEP10 Pooled Human Cryopreserved Hepatocytes; Thermo Fisher Scientific) were injected into the lower pole of the spleen. During the surgery, 1%-2% inhaled isoflurane was used for anesthesia, and 0.05-0.1 mg/kg subcutaneous buprenorphrine was used as needed for analgesia in the perioperative and postoperative periods. Following transplantation, NTBC was gradually withdrawn over several weeks according to the manufacturer's instructions, and human albumin levels in the blood were monitored on a monthly basis using the Human Albumin ELISA Quantitation Set (Bethyl Laboratories) according to the manufacturer's instructions, with the levels used to estimate % reconstitution with human hepatocytes.[2]

Chimeric liver-humanized mice that were 8 to 11 months of age (at least 5 months following transplantation) were used for experiments. After initial blood samples were collected, mice were administered 1×10^{11} particles each via retro-orbital injection. 1%-2% inhaled isoflurane was used for anesthesia at the time of the injections. A total of five mice were given CRISPR-*PCSK9* virus, and six mice were given CRISPR-control virus. As much as possible, the mice in the two groups were matched with respect to age and, to some degree, % reconstitution with human hepatocytes, though the mice with the best % reconstitution were preferentially used for the CRISPR-*PCSK9* group. After four days, the mice were sacrificed by carbon dioxide asphyxiation after overnight fasting. Whole liver samples were harvested for DNA analysis, and terminal blood samples were collected. Pre-treatment and post-treatment human PCSK9 levels in the blood were measured using the Human Proprotein Convertase 9/PCSK9 Quantikine ELISA Kit (R&D Systems) according to the manufacturer's instructions. Pre-treatment and post-treatment mouse PCSK9 levels in the blood were measured using the Mouse Proprotein Convertase 9/PCSK9 Quantikine ELISA Kit (R&D Systems) according to the manufacturer's instructions. Pre-treatment and post-treatment human albumin levels in the blood were measured using the Human Albumin ELISA Quantitation Set according to the manufacturer's instructions.

On-target and off-target mutagenesis analyses. To analyze the *PCSK9* exon 1 on-target site, liver genomic DNA samples were isolated and PCR amplicons for the on-target site were subjected to Surveyor assays as previously described.[1] PCR amplicons from two of the CRISPR-*PCSK9* mice were subjected to next-generation DNA sequencing at the Massachusetts General Hospital CCIB DNA Core (CRISPR Sequencing service; https://dnacore.mgh.harvard.edu/new-cgi-bin/site/pages/crispr_sequencing_main.jsp).

Off-target sites were predicted using the CRISPR Design server (http://crispr.mit.edu/)[3] and the COSMID server (https://crispr.bme.gatech.edu).[4] Seven of the top eight sites (all with a score > 0.1) from the list generated by the CRISPR Design server (Supplemental Figure I) as well as the single predicted off-target site from the COSMID server (Supplemental Figure II) were PCR amplified from two of the CRISPR-*PCSK9* mice and one of the CRISPR-control mice and subjected to next-generation DNA sequencing at the Massachusetts General Hospital CCIB DNA Core. The remaining site of the top eight sites from the list generated by the CRISPR Design server proved to have an extremely high background sequencing error rate and so was not analyzed for this study.

On-target and off-target mutagenesis rates were determined as previously described.[5] In brief, sequencing data were processed according to standard Illumina sequencing analysis procedures. Processed reads were mapped to the expected PCR amplicons as reference sequences using custom scripts; reads that did not map to reference were discarded. Frequencies of on-target and off-target indels were determined as follows. The reads were analyzed using custom scripts to identify indels by matching reads against reference, with indels involving any portion of the sequence within 15 nt upstream or downstream of the predicted CRISPR-Cas9 cleavage site (3 nt upstream of the 3' end of the protospacer) considered to be possible on-target or off-target effects. Reads for which there was any 18-nt sequence with more than 2 mismatches with the corresponding 18-nt portion of the reference sequence, either upstream or downstream of a candidate indel, were discarded as errors.

Primer sequences. Genomic amplification of on-target site: *PCSK9* exon 1: 5', CACGGCCTCTAGGTCTCCT; 3', GCCTCCCATCCCTACACC. Genomic amplification of off-target sites: OT1: 5', GGGAGGAAGAGCTGTGTGG; 3', AGCATCCTGGGTCATCAGAC. OT2: 5', CCAAAATGCCTTGAGCCTAA; 3', GGGGTATATGGCTTGGGAAC. OT3: 5', GGCATTAGAGAGGCAGTGGA; 3', TATCACAGTCCTGCCCACAA. OT4: 5', GCTGTTTGGTTCAGCAAATG; 3', GACCCCTTCTGGAAATGTGA. OT5: 5', GGGACCTACTGCACTCTTCG; 3', CCTGGTGACAGAGCGAGACT. OT6: 5', GACAGGGAGCAAACAGCTTC; 3', TAAATGGTTGGCGGTCTCAT. OT7: 5', CATCTTCCACTCCCACACCT; 3', CGATTGCCTATTTCTGAGCTG. OT8: 5', TGATCCTCTGACCACACCAA; 3', CAGATGGCAAACCCAACATT.

Supplemental References

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