REDUCTION OF CHOLESTEROL IN HUMANIZED MICE USING CRISPR/CAS9

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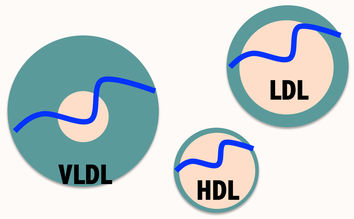
INTRODUCTION

CARDIOVASCULAR DISEASE (CVD)

CVD IS A CLASS OF DISEASES THAT INVOLVE THE HEART OR BLOOD VESSELS AND IS THE LEADING CAUSE OF DEATH GLOBALLY. HIGH CHOLESTEROL (HYPERCHOLESTEROLEMIA) IS A RISK FACTOR FOR THIS DISEASE, AND PREVENTATIVE MEASURES TAKEN TO LOWER CHOLESTEROL OFTEN DECREASE LIKELIHOOD OF THIS DISEASE. USING CRISPR/CAS9 GENE EDITING TECHNOLOGY, WE ATTEMPT TO DELETE MULTIPLE GENES INVOLVED IN CHOLESTEROL UPTAKE AND METABOLISM.

LIPOPROTEINS

FATS ARE TRANSPORTED THROUGH THE AQUEOUS ENVIRONMENT OF THE BLOOD THROUGH LIPOPROTEINS (I.E. LDL, HDL). APOB (BLUE) IS THE STRUCTURAL COMPONENT OF THESE PARTICLES.



FAMILIAL HYPERCHOLESTEROLEMIA (FH)

FH IS A DISEASE IN WHICH THERE ARE ABNORMALLY HIGH LEVELS OF LDL IN THE BLOOD, AND CAN BE CHALLENGING TO TREAT. PATIENTS WITH FH HAVE A MISSING LDL RECEPTOR, WHICH PREVENTS LDL CHOLESTEROL FROM LEAVING THE BLOODSTREAM AND ENTERING THE CELL.

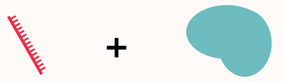
HUMANIZED MOUSE

WE CONDUCT OUR EXPERIMENTS ON A MOUSE MODEL THAT HAS ITS HEPATOCYTES (LIVER CELLS) REPOPULATED WITH HUMAN CELLS. THIS ALLOWS US TO TEST OUR GENE EDITING IN HUMAN CELLS, IN-VIVO.

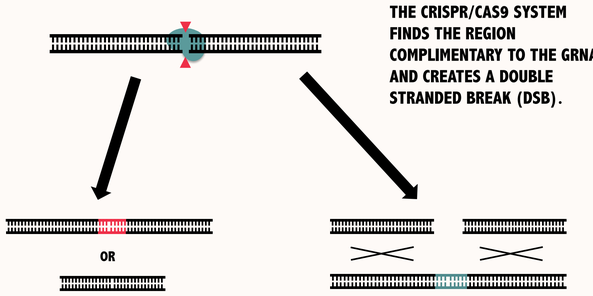
GOAL

OUR GOAL IS TO LOWER CHOLESTEROL BY KNOCKING OUT 4 GENES WITH CRISPR/CAS9 AS A POTENTIAL THERAPY

CRISPR/CAS9



THE CRISPR/CAS9 SYSTEM CAN BE SIMPLIFIED INTO TWO COMPONENTS: A GUIDE RNA THAT DIRECTS THE CAS9 TO A PRECISE REGION OF DNA, AND THE CAS9 PROTEIN THAT CUTS BOTH STRANDS OF DNA.



THE CRISPR/CAS9 SYSTEM FINDS THE REGION COMPLIMENTARY TO THE GRNA AND CREATES A DOUBLE STRANDED BREAK (DSB).

THE REPAIR MECHANISM IN MAMMALIAN CELLS FROM A DSB CONSISTS OF EITHER NON-HOMOLOGOUS END JOINING (NHEJ) OR HOMOLOGY DIRECTED REPAIR (HDR)

NHEJ INTRODUCES VARIABLE LENGTH INSERTIONS OR DELETIONS (INDELS) IN ATTEMPT FOR THE CELL MACHINERY TO CONNECT THE NON-HOMOLOGOUS ENDS OF THE DNA STRAND. THESE INDELS ULTIMATELY RESULT IN THE DISRUPTION OF THE GENE OF INTEREST

HDR CAN BE USED TO INTRODUCE A NEW GENE INTO THE REGION THROUGH TARGETED RECOMBINATION. THIS TECHNIQUE USES LONG STRETCHES OF HOMOLOGOUS DNA ON BOTH SIDES OF THE GENETIC SEQUENCE BEING INTRODUCED INTO THE GENOME.

THERAPEUTIC KNOCKOUT TARGETS

APOB

THE APOB GENE CODES FOR APOLIPOPROTEIN B, THE STRUCTURAL COMPONENT OF LDL PARTICLES. APOB IS SYNTHESIZED IN THE LIVER AND BINDS TO THE LDL RECEPTOR (LDLR) TO ALLOW LDL ENTRY INTO THE CELL.

FDFT1

THE FDFT1 GENE CODES FOR THE ENZYME SQUALENE SYNTHASE. THIS ENZYME CATALYZES THE SYNTHESIS OF SQUALENE, A PRECURSOR TO CHOLESTEROL SYNTHESIS.

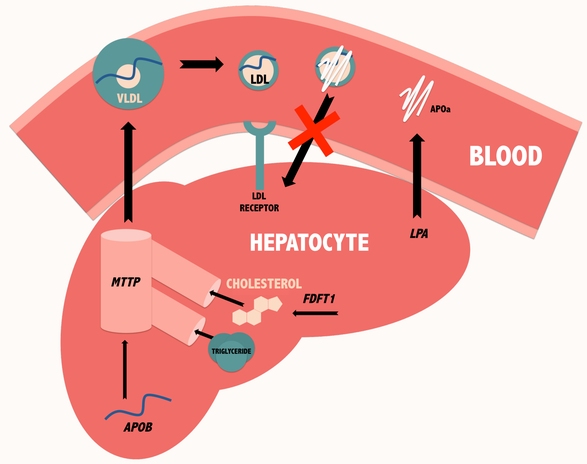
MTTP

THE MTTP GENE CODES FOR A PROTEIN CALLED MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN. THE MAIN ROLE OF MTTP IS TO ASSEMBLE LIPOPROTEINS.

LPA

THE LPA GENE CODES FOR APOLIPOPROTEIN(A), WHICH BINDS TO A NORMAL LDL PARTICLE TO FORM LIPOPROTEIN(A). LIPOPROTEIN(A) CANNOT ENTER THE CELL VIA LDLR AND IS ASSOCIATED WITH CARDIOVASCULAR DISEASE.

ROLE OF GENE TARGETS ON LIPOPROTEIN METABOLISM



OBJECTIVES

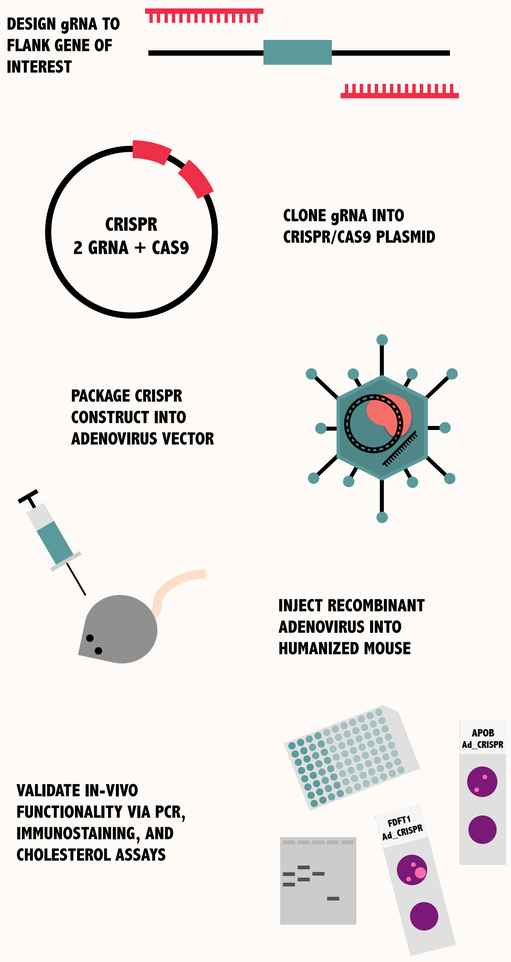
THE SPECIFIC AIMS OF THIS EXPERIMENT ARE:

1. DESIGN AND PACKAGE A CRISPR/CAS9 CONSTRUCT INTO AN ADENOVIRUS-5 VECTOR
2. ASSESS FUNCTIONALITY OF AD5_CRISPR VECTOR IN VITRO
   - TRANSDUCE HUH7 HEPATOCELLULAR CARCINOMA CELL LINE
   - ASSESS TOXICITY
   - GENOMIC DELETION OF TARGET GENES
3. KNOCKOUT FOUR CHOLESTEROL-REGULATING GENES IN-VIVO USING THE AD5_CRISPR SYSTEM IN A HUMANIZED MOUSE
   - OBSERVING A CHOLESTEROL LOWERING EFFECT IN A HUMANIZED MOUSE
   - ASSESS FUNCTIONALITY IN-VIVO
   - MEASURE TOXICITY
   - OBSERVE CHOLESTEROL LEVELS IN MICE WITH HIGH FAT DIET THAT HAVE BEEN TREATED WITH AD5_CRISPR VIRUS.

MATERIALS AND METHODS

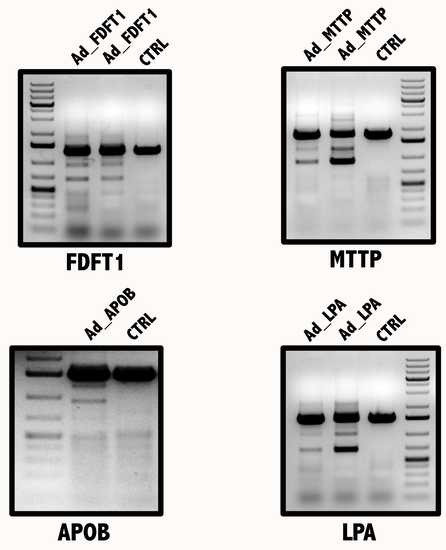
- TWO GUIDE RNA MOLECULES WERE DESIGNED TO CUT INSIDE THE INTRON SURROUNDING A CRITICAL EXON OF EACH TARGET GENE.
- GUIDE RNAS WERE CLONED INTO A CRISPR VECTOR CONTAINING TWO U6 PROMOTERS FOR EACH GRNA AND A LIVER SPECIFIC TBG PROMOTER FOR THE CAS9 PROTEIN.
- THE CRISPR PLASMID WAS PACKAGED INTO AN ADENOVIRUS-5 VECTOR BY THE VECTOR DEVELOPMENT LABORATORY AT BAYLOR COLLEGE OF MEDICINE.
- RECOMBINANT ADENOVIRUS CELL LYSATES AND PURIFIED VIRUS WERE TRANSDUCED INTO HUH7 CELLS AND INCUBATED FOR 72 HOURS.
- CELLS WERE HARVESTED AND A PCR WAS DONE WITH PRIMERS DESIGNED TO SURROUND DELETED EXONS.

EXPERIMENTAL DESIGN



RESULTS

PCR of Ad_CRISPR INFECTED HUH7 CELLS



HUH7 CELLS WERE GROWN TO 70% CONFLUENCE AND TRANSDUCED WITH PURIFIED RECOMBINANT ADENOVIRUS. A MULTIPLICITY OF INFECTION (MOI) OF 1000 AND 1500 VIRAL PARTICLES PER CELL WAS USED. THE HEAVY UPPER BANDS CORRESPOND TO THE UNEDITED, WILD-TYPE GENE WHILE THE LOWER BANDS CORRESPOND TO THE SUCCESSFUL EXCISION OF THE GENE OF INTEREST.

FUTURE DIRECTIONS

INJECT LARGE SCALE PREP OF RECOMBINANT ADENOVIRUSES INTO SEVERAL MICE ON A HIGH FAT DIET

ASSESS TOXICITY AND FIND THERAPEUTIC DOSE FOR POSSIBLE GENE THERAPY TREATMENT

ASSESS LONG TERM EFFECT OF GENE KNOCKOUTS IN HUMANIZED MICE

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