Optimisation of transfection methods using DNA and Protein formats for CRISPR Cas9 mediated gene knock out in Beta-TC-6 cells.

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INTRODUCTION

Beta-Tumour cells (βTC) are a group of highly differentiated beta cell lines derived by expression of the SV40 T antigen (Tag) oncprotein under control of the insulin promoter in transgenic mice. 

βTC-6 cells exhibit glucose stimulated insulin secretion which makes them a valuable tool in understanding the mechanisms that regulate insulin secretion.

CRISPR/Cas9

Recently emerged as a powerful and highly efficient genome engineering tool. Provides new approaches for generating in vitro disease models presenting an opportunity to study rare genetic diseases. ² ³

CRISPR/Cas9

Design of gRNAs to disrupt the ABCC8 gene

RESULTS

Optimisation of Transfection using GFP plasmid

MATERIALS AND METHODS

Gene editing by CRISPR/Cas9

Delivery methods for delivery of CRISPR/Cas9 and gRNA to the βTC6 cells

Cas9+gRNA as an RNP complex:

ø No transcription of RNA or translation of proteins
ø Transient nature → fewer off target effects

CONCLUSIONS AND FUTURE WORK

Conclusions:
- Design of gRNAs to target areas of Abcc8
- Cloning of gRNAs into Cas9 plasmid
- Transfections of βTC 6 with the Cas9 + gRNA plasmid & RNP complex

Future Work:
ø Optimising the delivery of Cas9/gRNA system using electroporation
ø gRNA-RNP complex
ø gRNA plasmid
ø Molecular validation of the KO Abcc8 model
ø T7 ENDO assay
ø Sanger sequencing

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References