CO-TARGETING HYPOXANTHINE-GUANINE PHOSPHORIBOSYLTRANSFERASE TO INCREASE KNOCKOUT EFFICIENCY WITH CRISPR/CAS9 RIBONUCLEOPROTEINS IN PATIENT-DERIVED COLORECTAL CANCER ORGANOIDS

Michael Boutros

UEG Week Copenhagen 2023 | Poster

Colorectal

October 17, 2023

A co-targeting strategy using CRISPR/Cas9 RNPs against HPRT followed by 6-thioguanine selection significantly increases knockout efficiency in patient-derived colorectal cancer organoids.

KEY POINTS

- Co-targeting HPRT alongside a gene of interest with CRISPR/Cas9 RNPs, followed by 6-thioguanine treatment, enriches for cells with both HPRT knockout and the desired knockout by selecting for 6-thioguanine-resistant HPRT-deficient cells.
- An optimized 384-well CRISPR/Cas9 RNP transfection platform achieved 40-60 percent genome editing efficiency in adherent cell lines 48 hours after transfection, which was further increased by the HPRT co-targeting and 6-thioguanine strategy.
- In patient-derived CRC organoids, optimized electroporation with CRISPR RNPs achieved knockout efficiencies of more than 40 percent by FACS analysis for surface markers CD81, CD46, and CD151, and 20 percent by Sanger sequencing at individual guide target sites.
- The method enables high-efficiency knockouts in patient-derived organoids without lentiviral transduction, and CRISPR RNPs offer quicker editing and lower off-target rates compared to DNA-based CRISPR delivery.
- This approach is intended for validating genes identified in arrayed CRISPR screens and may support development of targeted therapies for colorectal cancer.

VIEW THIS CONTENT ON GUTFLIX

https://gutflix.eu/search/d/275d930a-743d-11ee-9add-0242ac140004

AI-generated summary

This summary was generated by an AI large language model based on the content transcript. It is for informational purposes only and should not be considered a substitute for clinical judgment. Always rely on your professional expertise and the full clinical context when making clinical decisions.